

Developmental Process	NF stage number	NF stage name	LANDMARKS		
			EXTERNAL	INTERNAL	MOLECULAR MARKERS (<i>gene</i> : anatomy)
FERTILIZATION	stage V oocyte	oocyte	1 cell; ovulated unfertilized egg; animal hemisphere dark, vegetal hemisphere light; animal-vegetal axis in random orientation; soft texture	large nucleus, 'germinal vesicle' in animal hemisphere	<i>nanos1</i> : mitochondrial cloud; <i>vegt</i> : vegetal cortex
	1	1-cell, fertilized egg	1 cell, vitelline membrane swells; eggs rotate with dark animal hemisphere up, light vegetal hemisphere down; firm texture; sperm entry point indicated by pigment condensation; germinal vesicle breakdown leaving pale spot in animal hemisphere	cleavage has not begun; germinal vesicle breaks down and pronuclei fuse; visible cortical layer thickest on animal and dorsal sides.	
CLEAVAGE	2-		first cleavage begins as a top to down furrow in animal hemisphere	cleavage furrow has not yet reached the vegetative pole	
	2	2-cell	2 cells; 1st cleavage plane is meridional along the dorso-ventral plane dividing right and left halves		<i>atp4a</i> : animal hemisphere; <i>vegt</i> : vegetal hemisphere
	3	4-cell	4 cells; 2nd cleavage is meridional, perpendicular to the 1st cleavage; in many embryos, the 2 dorsal blastomeres are smaller and lighter (on animal surface) than 2 larger darker ventral blastomeres [1]	cleavage cavity present	
	4	8-cell	8 cells; 3rd cleavage plane is equatorial giving 4 smaller animal blastomeres (micromeres) and 4 larger vegetal blastomeres (macromeres); animal dorsal cells are lighter and ventral cells are darker in some embryos		<i>nanos1</i> : germ plasm
	5	16-cell	16 cells; 4th cleavage is again meridional; animal blastomeres smaller than vegetal; dorsal blastomeres lighter than ventral.		
	6	32-cell	32 cells; 5th cleavage equatorial, giving 4 rows each with 8 blastomeres; animal pole with smallest rosette of microblastomeres, 2 middle rows of irregular shaped cells, larger vegetal rosette of macromeres		
	6.5	morula	64 cells; 6th cleavage; cleavages becoming asynchronous; animal/dorsal blastomeres divide before vegetal blastomeres		<i>gdf1</i> : vegetal hemisphere; <i>atp5f1a</i> , <i>h3-3a</i> , <i>shroom1</i> , <i>shroom2</i> : animal hemisphere
BLASTULA	7	early blastula	128-512 cells; 7th-9th cleavages, no longer possible to count cells reliably; size of animal cells can distinguish embryo stage	pre-gastrulation cell movement begins; internal blastocoel cavity forms	<i>nr5</i> : earliest zygotic transcription
	8	middle blastula	~1000-4000 cells; 10-12+ cleavages; cell number no longer a stage guide; animal hemisphere with small dark pigmented cells referred to as 'animal cap'; animal surface looks 'pebbly'	at stage 8.5, after 12th cell divisions the mid-blastula transition (MBT) occurs; asynchronous internal cell division; zygotic genome activation	<i>gs17</i> , <i>nr1</i> : major initiation of zygotic transcription
	9	late blastula	animal hemisphere still darkly pigmented; animal surface more like 'grains of sand' than 'pebbles' of NF stage 8	blastocoel now maximum size; 3 germ layers becoming distinct; ectoderm in the animal 1/3 of the embryo, a ring of mesoderm in the middle and yolk endoderm on the vegetal 1/3	<i>sox17</i> : endoderm; <i>tbxt</i> : mesoderm
GASTRULATION	10	initial gastrula	darker pigment from bottle cells on the dorsal vegetal surface indicate dorsal blastopore lip formation, between '11 & 1 o'clock'; blastopore groove where cell ingress, is not yet visible	initial formation of Spemann-Mangold Organizer at the dorsal blastopore lip	<i>gsc</i> : dorsal blastomere lip; <i>tbxt</i> : marginal zone
	10.25	early gastrula	blastopore lip extends laterally ~1/4 around the circumference, from ~'10 to 2 o'clock'; indentation of blastopore visible as a groove	marginal zone involutes on dorsal side; internal dorsal mesendoderm begins to migrate anteriorly along blastocoel surface; vegetal rotation of endoderm mass	
	10.5	crescent-shaped blastopore	arch of pigmented blastopore lip extends laterally from dorsal to lateral sides ~1/4 to 1/2 around the circumference; yolk plug almost round	medial-lateral intercalation of dorsal mesoderm; 'Brachet's cleft' forms between internal leading edge mesendoderm and the dorsal mesoderm; neural induction begins, dorsal-ventral patterning	<i>chrd.1</i> : dorsal mesoderm; <i>ventx2</i> , <i>wnt8a</i> : ventro-lateral mesendoderm; <i>hhex</i> : anterior mesendoderm; <i>tbxt1</i> : involuted dorsal mesoderm
	11	horse-shoe shaped blastopore	pigmented blastopore lip ~ half the circumference, pigmented bottle cells extend to the ventral side; yolk plug ~1/2 diameter of embryo, is slightly elongated in the dorsal-ventral direction	Brachet's cleft begins to open forming the archenteron; ectoderm, mesoderm and endoderm germ layers specified; blastocoel becoming smaller, mesodermal mantle undergoes convergent extension and endoderm is internalized	<i>sox17</i> : endoderm; <i>tbxt</i> : mesoderm; <i>sox2</i> : neurectoderm; <i>krt12.4</i> : non-neural ectoderm
	11.5	large yolk plug	blastopore lip extends all the way around yolk plug; ventral blastopore lip more darkly pigmented; yolk plug not quite round, ~1/3 of embryo diameter elongated in the dorsal-ventral direction	blastocoel displaced to the ventral side; involuting mesendoderm mantle continues to extend anteriorly	
	12	medium yolk plug	areas of light and dark pigment radiating from yolk plug (flower petal like); yolk plug small and circular, a little less than 1/4 of egg diameter, diameter decreasing; neurectoderm transforming into the discernible neural plate	blastocoel begining to close as the archenteron expands	<i>sox2</i> , <i>sox3</i> : neural plate
	12.5	small yolk plug	darker pigment lines on dorsal surface indicate future median groove and neural plate; blastopore slit is slightly open, diameter decreasing and oval shaped	clear delineation of the 3 primary germ layer tissues; notochord forms from mediolateral convergence of dorsal axial mesoderm under the neural ectoderm	<i>chrd.1</i> , <i>nog</i> , <i>shh</i> : axial mesoderm, notochord
	13	slit blastopore	blastopore completely closed to a 'slit'; neural plate on dorsal side clearly outlined	neural crest form at the edge of the neural plate, i.e., the boundary between the neural and non-neural ectoderm	<i>sox2</i> , <i>sox3</i> : neural plate; <i>dlx3</i> , <i>msx1</i> : neural plate border; <i>snail2</i> : neural crest; <i>tbx2</i> : cement gland primordium; <i>myf5</i> : paraxial mesoderm; <i>rax</i> : optic field
	13.5	initial neural plate	sharp demarcation of anterior neural plate; anterior aspect of neural plate bent down; yolk plug internalized		<i>tubb2b</i> : differentiating neural progenitors; <i>foxf1c</i> : anterior neural plate border
	14	neural plate	neural plate obvious; dorsal midline thin with neural folds thickening anteriorly and laterally; neural folds begin to elevate; convergence extension begins to narrow neural plate posteriorly	blastocoel continues to close in the ventral foregut region as archenteron expands on the internal dorsal side	<i>pax6</i> : eye field, neural plate, lens placode; <i>pax2</i> : between anterior and posterior neural plate
NEURULATION	15	early neural fold	neural folds distinct; anterior neural fold round; demarcation of neural plate clear caudally, narrowing in middle and caudal regions; neural groove deepens; pigmented cement gland primordium faintly visible at the ventral-anterior border of neural plate, cells stand out from epithelial layer.	physical segregation of neural crest from anterior neural plate; blastocoel closes in the ventral foregut	<i>pax3</i> : neural fold/neural crest; <i>pax8</i> : intermediate mesoderm; <i>rax</i> , <i>otx2</i> , <i>eyaf1</i> , <i>six1</i> : optic field; <i>hhex</i> : foregut endoderm
	16	mid-neural fold	eye primordia [2] become discernible as indentations near the lateral edges of the anterior neural plate; anterior neural plate 'rectangular' in shape; neural plate sharply constricted in the middle.	right and left cardiac mesoderm migrates to anterior-ventral midline; foregut diverticulum forms	<i>pax6</i> : optic field, anterior neural plate, lens placode; <i>nkx2-5</i> : cardiac progenitors; <i>krt12.4</i> : ectoderm, non-neural ectoderm, epidermis

GUT-COILING		42	free swimming tadpole	opercular fold first visible; head somites I and II disappeared	trachea and esophagus separate				<i>sox2</i> : esophagus and stomach; <i>nkx2.1</i> : trachea and lung buds; <i>sftpc</i> : lung buds
		43	free swimming tadpole	cement gland starts to lose pigmentation; lateral line pits visible	stomach has lengthened further; pancreas shifted to right side; duodenum formed by 1st gut coil constriction to anterior-right; midgut + hindgut form hairpin curve, visible on left side (will become the 'apex' of future intestinal coil)				<i>foxl1, spdef, bmp1a, cfap161</i> : stomach
		44	free swimming tadpole	heart fully formed and clearly visible; tentacles start to grow; gills shrinking	septum begins to form in heart atrium which is slightly anterior to ventricle; midgut and hindgut lengthened more; the intestinal apex visible in ventral view as a "U" shape in the upper left quadrant of the gut cavity	visual avoidance behavior begins			<i>tnni3, frzb, sox9, nkx2-5, actc1, cellf1</i> : heart; <i>apoe, cdx2, gpd2, jund</i> : midgut-hindgut
		45	feeding tadpole [3]	operculum partly covers the gills, hindlimb bud not visible	midgut and hindgut continue to lengthen; the intestinal apex begins to rotate inward in a counterclockwise trajectory; spleen forms; mesonephric kidney	tadpoles to start swim continuously and begin feeding			<i>pdlim5, darmin</i> : hindgut; <i>a2m</i> : midgut, hindgut, liver
PREMETAMORPHOSIS		46	feeding tadpole	crescent-shaped hindlimb bud first appears; pigment cells appear on eye and around abdomen; trunk somite 1 disappeared	midgut and hindgut lengthen further- apex continues to rotate inward, forming multiple coils of intestine; blood circulation to gills diminishing	food can be seen in intestine; now feeding			
		47	feeding tadpole	iridescent gold-coloured abdominal wall surrounds coiled gut; blood circulation visible from heart to gills, and through paired dorsal aorta; cement gland starts to degenerate; tentacles longer	retinal ganglion cells have formed complex synapses with optic tectum neurons; thyroid gland begins to function; thymus primordium detectable			<i>foxn1</i> : thymus primordia	
		48		hindlimb bud shape nearly semi-circular	retinal ganglion cells-optic tectum synapses more compact				<i>fgf8, fgf2, salil4</i> : hindlimb bud
		49		hindlimb bud length equal to it's width	thyroid follicles first appear				<i>foxe3, nkx2-1, pax2</i> : thyroid
		50		hindlimb bud slightly constricted at base; tiny oval forelimb buds just visible	gonads undifferentiated				<i>fgf2</i> : forelimb bud; <i>spry4</i> : hindlimb bud
		51		hindlimb is cone-shaped; forelimb bud is oval shaped (in lateral view)	resorption vacuoles in thyroid follicles first appear				
		52		hindlimb bud with slight 'wrist' indent; forelimb bud slightly constricted at base	5 complete coils of the intestine	regeneration competent [4]			<i>sox9</i> : hindlimb digits/cartilage elements
		53		hindlimb paddle-like, with wrist constriction, digits not discernable; forelimb limb bud with slight wrist constriction	onset of sexual differentiation of gonads	regeneration competent; thyroid animals have arrested development			
		54		hindlimb length (not including foot) 2x the width; foot paddle splayed with 5 digits and thinner inter-digital webbing; forelimb paddle with 4 digits and thinner inter-digital membranes	pronephric kidney begins to atrophy	thyroid hormone detectable in blood			<i>tbx4, salil4</i> : interdigital mesenchyme
		PROMETAMORPHOSIS		55		hindlimb length (not including foot) 3x width; forelimb hand rotates 90 degrees, free parts of fingers as long as they are wide	all major muscles of hindlimb developed	regeneration restricted [4]	
56				hindlimbs visible from above as they can rotate away from body; hindlimb length = ~ 5 tail somites	sexual differentiation of gonads; hindlimb skeleton completely chondrified	regeneration restricted			
57				hindlimb length = ~ 9 tail somites; forelimb remains enclosed in operculum; lip folds form					
58				hindlimb length = 11-12 tail somites; claws form on toes 1-3 (mostly always still white); forelimb emerges from operculum, elbows first; tail tip begins to atrophy	melanin/pigment deposited under skin especially in tail	regeneration incompetent [4]			
59				hindlimb muscular, claws start to harden and turn black, shortest toes first; fingertips reach base of hindlimb when forelimb is positioned along the abdomen; tentacles/barbels regress	melanin/pigment surrounds blood vessels and between fibres of somites; forelimb muscles differentiated; pronephros no longer functional	regeneration incompetent			
60				gill chamber opening still wide; fingertips reach beyond base of hindlimb (almost to 'knee') when forearm is positioned along side of the abdomen; forelimb held posterior to heart; tail fins greatly reduced	pigmentation across body increases	regeneration incompetent; animal switches from tail to leg swimming			
61				first sign of gill resorption, openings to gill chamber much narrower; hindlimb and forelimb fully formed; forelimb at level of posterior half of heart	lateral finger-like protrusions from olfactory organ	cessation of feeding (due to oral and intestinal remodeling)			
CLIMAX OF METAMORPHOSIS		62	tailed froglet	head slightly broader than abdomen: corner of mouth still in front of eye; forelimb reaches middle of heart; ventral fin gone from abdomen; adult skin on hindlimbs; only tiny nubs of tentacles remain.	tiny 'stirnorgan' (light detecting cells/part of pineal gland) appears; notochord atrophies along length of tail	peak levels of thyroid hormone in plasma			
		63	tailed froglet	head narrower than abdomen: tentacles (most often) completely gone; forelimb at level of anterior half of heart; tail shortens as somites are rapidly resorbed, tail still slightly longer than body					
		64		corner of mouth behind eye; tail length is about 1/3 of body length, at level of ankle when legs are in typical neutral position; body completely covered in adult skin, but 'border lines' clearly visible	thymus gland ventral-lateral to otic capsule				
		65		tail length a few millimeters, all tail somites have disappeared; body completely covered in adult skin, but 'border lines' still visible in some areas		feeding resumes			
		66	froglet	tail very nearly gone, not visible from ventral view; adult skin 'border lines' have disappeared, froglet body ~ 10mm long	skin remodelled with underlying dermis and secretory glands	thyroid hormone in plasma returns to prometamorphic levels			

FOOTNOTES

- Dorsal-ventral pigment variation only occurs in some batches of embryos. Select 2-4-cell embryos with clear pigment variation - otherwise only accurate about 70% of time.
- In the Normal Table and other texts, organ primordia are often called 'anlage'; they are visualized by as a thickening of specific cells via histology or by molecular markers. Search specific XAO terms on Xenbase for more molecular markers
- NF stages 41-66 are not referred to as having specific 'stage names' by Nieuwkoop and Faber
- Regeneration classes from Aztekin et al 2021 PMID:34116722.