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Development of Digestive System of Indus Valley Toad, *Bufo stomaticus* Lütken

MUHAMMAD SHARIF KHAN*
Herpetological Laboratory, Rabwah 35460, Pakistan
E-mail: Typhlops99@hotmail.com

Abstract.- Development, growth and metamorphosis of the digestive system of Indus Valley toad *Bufo stomaticus* is followed. Intrinsic and extrinsic factors affecting development are discussed.

Key words: *Bufo stomaticus*, morphogenesis.

INTRODUCTION

Exotroph anuran amphibians are unique among tetrapods because of their biphasic life style. The embryo develops into an independent aquatic self feeding non breeding tadpole phase, which metamorphosis into adult breeding phase (Slade and Wassersug 1975; Wassersug 1975a, b). Tadpole's digestive system starts developing soon after neural tube is established in the embryo (Khan, 1965, 1969) becomes spirally coiled and functional during tadpole phase. During metamorphosis programmed cell deaths brings about regression in the size of digestive tract to convert it into adult's shorter digestive system (Fox, 1970, 1981; Nakajima *et al*., 2005).

External morphological changes occurring during normal development of different amphibian species are available in tabular form (Cambar and Marrot, 1954; Del Conte and Sirlin, 1952; Gallien and Houillon, 1951; Nieukoop and Faber, 1956; Khan 1965, 1969; Shimizu and Ota, 2003; see Khan, 1965 for list of older publications). Though exotroph anurans live in diverse habitats and ecological situations, they pass through parallel morphological changes during their development (Altig and Johston, 1989; Khan, 1991; McDiarmid and Altig, 1999; Khan and Malik, 1987). Khan (1965) compared developmental stages of *B. stomaticus* with other species listed above.

Present report presents stage by stage record of development of digestive system of *Bufo stomaticus* tadpole (Khan, 1965), from pre tadpole Stages (21-24), tadpole (25-35) till completion of metamorphosis (36-43).

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* Present address: 306 N. Morton Avenue, Morton, PA, 19070, USA.

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MATERIALS AND METHODS

Collection of material

The Indus Valley riparian system in Pakistan is inhabited by six sympatric anuran species: *B. stomaticus*, *Euphlyctis cyanophlyctis*, *Fejervarya limnocharis*, *F. syhadrensis*, *Hoplobatrachus tigerinus* and *Microhyla ornata* (Khan, 1968, 1976, 1982, 1994, 2002). They use same temporal ponds and puddles for breeding during monsoon rains, June-August (Khan, 1969; Khan and Tasnim, 1987; Khan and Malik, 1987). *Bufo stomaticus* eggs and tadpoles does not develop normally in laboratory (Khan, 1965), so that material for the present study were collected from temporal breeding sites lying within a radius of one mile around Rabwah city (31° 50' N, 72° 55' E), Punjab, Pakistan.

The tadpoles are fixed and preserved in diluted Bouin's fluid (1:1 water), as suggested by Khan (1965, 1969, 1991). *Bufo* tadpoles were identified by using key in Khan (1982), and staged with the help of Khan (1965).

Surgical procedure

Binocular dissection microscope was used for dissection, identification and surgery. A large Petri-dish with melted black wax was used to fix the tadpole during surgical procedure. Digestive tract is visible through transparent body wall at Stages 21-

23, however, during subsequent stages body wall thickens to obstruct the view, and hence needs surgery.

A three mm wide, 5 mm long shallow groove was made in the centre of the dish. The tadpole was placed abdomen up in the groove, held in position by an entomological pin passing vertically through tail just near the cloacal aperture. Devacker's angular spring irredectomy scissors were used. A longitudinal at tadpole's abdomen was made, saving underlying intestine from injury by lifting up the body wall with the help of fine tipped forceps. The intestinal spiral was gently lifted to observe and figure disposition of its different parts, which were figured with the help of a Camera Lucida.

No appreciable difference was noted in morphology of the tadpoles at same stage coming from different parents and collection sites.

DESCRIPTION

Development of tadpole's digestive system is divisible in three distinct periods:

I. Pre-larval: development of prototype from embryonic endodermal cellular mass (Stage 21-23).
II. Larval: development of digestive system with sinistral and dextral coils (Stages 24-37).
III. Metamorphosis: shortening of gut (Stages 38-43).

Stage numbers and stage criteria referred to in the following description correspond to those given in Khan (1965).

Pre-larval development

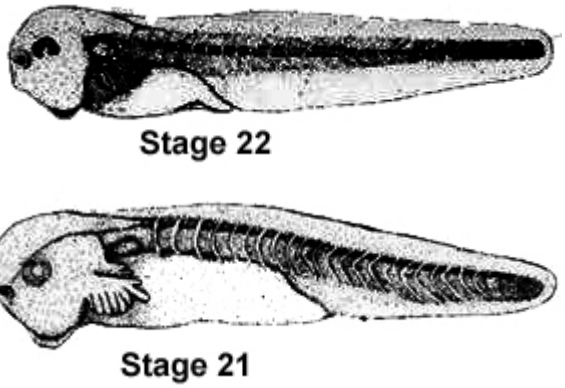
Stage 21 (Fig. 1)

Stage criteria: (Gill filament: tail-bud stage), analage for eye, first and second gills, narial pit appear; cement-gland is divided in two moieties; pronephric area becomes marked; tail-bud is marked.

Stage 22 (Fig. 2)

Stage criteria: (Three gill: fin stage), third gill with filaments; eye cups "C"-shaped; tail as long as body, with fins.

Digestive system: Stomodium a slit; pancreatic anlage marked 7 gastro-duodenal flexion (groove) 5; the ilio-cloacal flexion (groove) 6 deepens to shaped out large intestine 8; the endodermal cellular mass bulges to left 2.



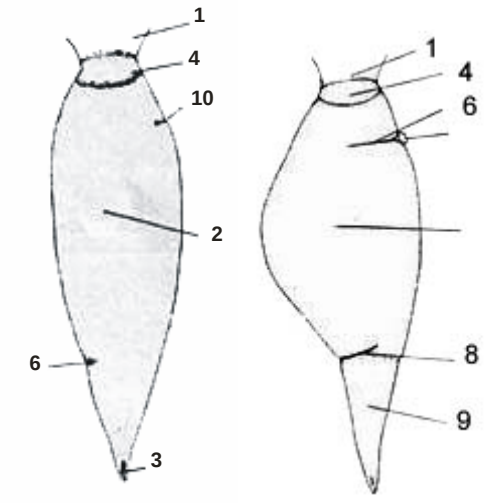


Fig. 1. Digestive system at Stage 21.
Fig. 2. Digestive system at Stage 22.
1, esophagus; 2, yolk (endodermal mass); 3, cloacal pit; 4, liver analage; 5, gastro-duodenal flexion; 6, ilio-cloacal; 7, pancreas; 8, large intestine; 9, stomach; 10, gastro-duodenal groove; 11, main coil; 12, gall bladder; 13, descending (anticlockwise) spiral; 14, ascending (clockwise) spiral; 15, loop of coil formation; 16, bile duct; 17, gastro-duodenal flexion; 18, duodenum; 19, pancreatic duct; 20, cloacae; 21, urinary bladder; 22, duodeno-iliac loop; 23, ilio-cloacal flexion; 24, cloacal tube; 25, hepato-pancreatic duct; 26, esophageo-gastric flexion; 27, ileum.

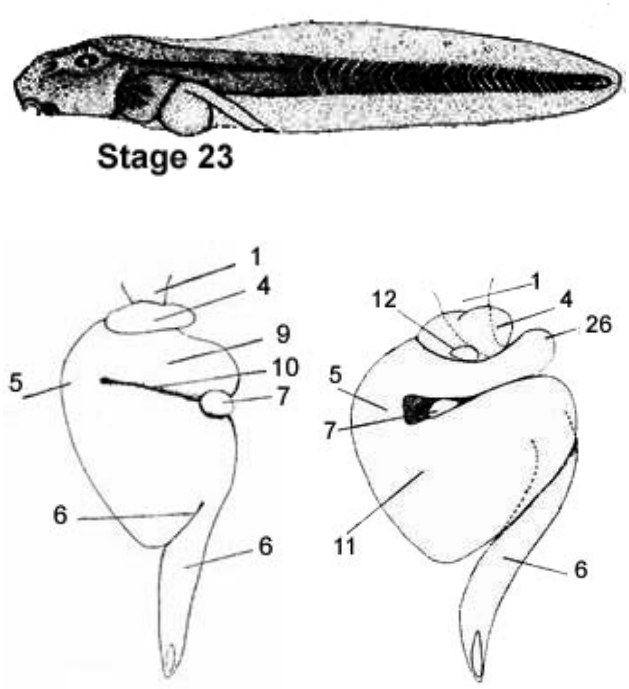
Digestive system: The stomodial pit is lined by four oblique ridges (jaw analages). Esophagus is marked 1; position of the cloacal aperture is marked

3; liver analage is marked 4; gastro-duodenal groove at anterior right side 10 and a ilio-cloacal groove at posterior left side 6 of the endodermal mass.

Stage 23 (Figs. 3, 4)

Stage criteria: (Opercular folds). Head becomes massive and dorso-ventrally depressed; opercular fold appear at the anterior base of the first gill; eye-ring and nasal openings become distinct; fourth gill appears; pronephric area disappears; body wall distinct; melanophores appear on head, body and tail; lateral line sense organs are indicated.

Digestive system: Liver (4) transversely enlarged and bilobed (Figs. 3, 4); gastric region is distinguished (9), become continuous with esophagus through a distinct esophageal-flexion (26); the gastro-duodenal groove widens to enclose pancreas (7: Fig. 5); the endodermal mass of cells is distinguished into descending (anti-clockwise) coil (11) by elongation of the tubular large intestine (8).



Figs. 3 and 4. Digestive system at Stage 23.
For abbreviations see Fig. 1.

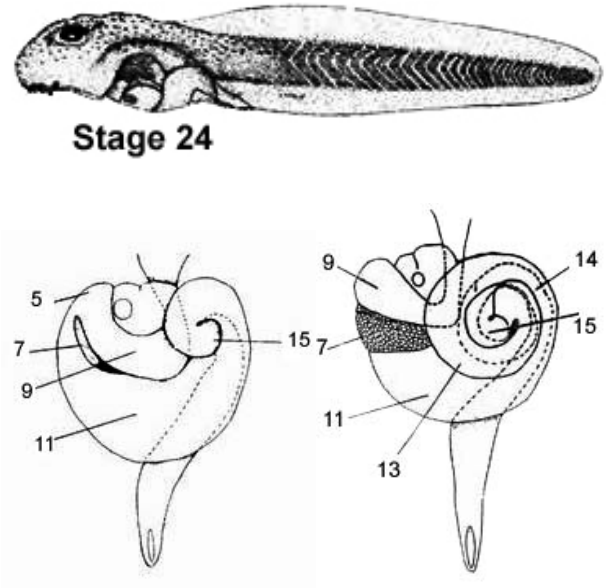
Larval development

Stage 24 (Figs. 5, 6)

Stage criteria: (Full operculum, oral sucker). Gills covered; spiracle distinct; tail exceeds body in length, body as broad as head.

Digestive system: Dark brown horny serrated oral ridges; labial keratodont formula 1,1+1/2; stomach (9) distinguished in three regions: (a) dorso-ventral proximal, cardiac part, continues rostrally with esophagus; (b) middle, gastric part, curves down left; (c) distal, pyloric part (11), curves right upward to initiate anticlockwise (descending) coiling of the intestine. Distally forms a sharp loop of coil formation (15), initiating clockwise coil (14), which merges into large intestine (8) opening distally at cloacal aperture. At this stage anti- and clock-wise spirals have 1+1/2 coils.

The liver is bilobed, with distinct gall bladder and bile duct.



Figs. 5 and 6. Digestive system at Stage 24.
For abbreviations see Fig. 1.

Stage 25 (Fig. 7)

Stage criteria: (Hind limb bud stage).

Digestive tract: Keratodonts formula is typical bufonid 1,2+2/3. Feeding starts, intestinal coils filled with food; fecal matter starts expelling through cloacal aperture.

2+1/2 coils in anti- (13) and clock-wise (14) spiral (Figs. 7, 8), piling over each other, joined in the center by the loop of coil formation.

The gyre of the outer intestinal spiral displaces stomach, liver and pancreas more to the left anterior side (Fig. 9).

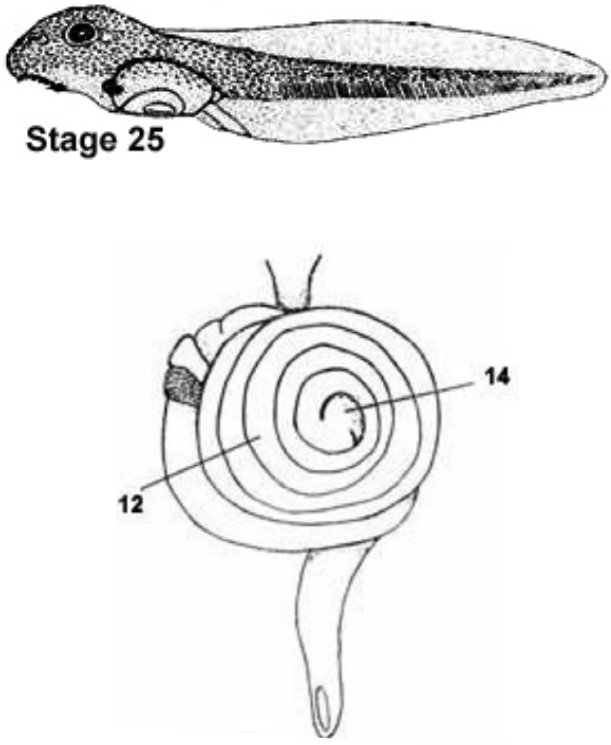


Fig. 7. Digestive system at Stage 25.
For abbreviations see Fig. 1.

Stages 26-37 (Fig. 8)

Stage criteria: Tadpole active, reacts sharply to stimuli. Hind limbs differentiated into thigh, shank and toes, with rudiments of web; limb shows movements, are used for support against vegetation when in shallow marginal water, where tadpole spends most of its time.

Metamorphosis

Stages 38-40 (Fig. 9)

Stage criteria: Tadpole becomes sluggish, stops feeding, mostly stays hiding in the marginal shallow water among vegetation**;** tail fins and cloacal tube disappear; cloacal opening is deflected to left side; fore limbs are visible through atrium; optic lens distinct; thighs make a angle of 75° with the body, foot clasped along the sides of the tail ventral fin; abdominal muscles develop and body wall becomes opaque.

Digestive system: Stage 37 marks maximum development of intestinal spirals 7-9 coils, entire gut measures 119 to 125 mm in length.

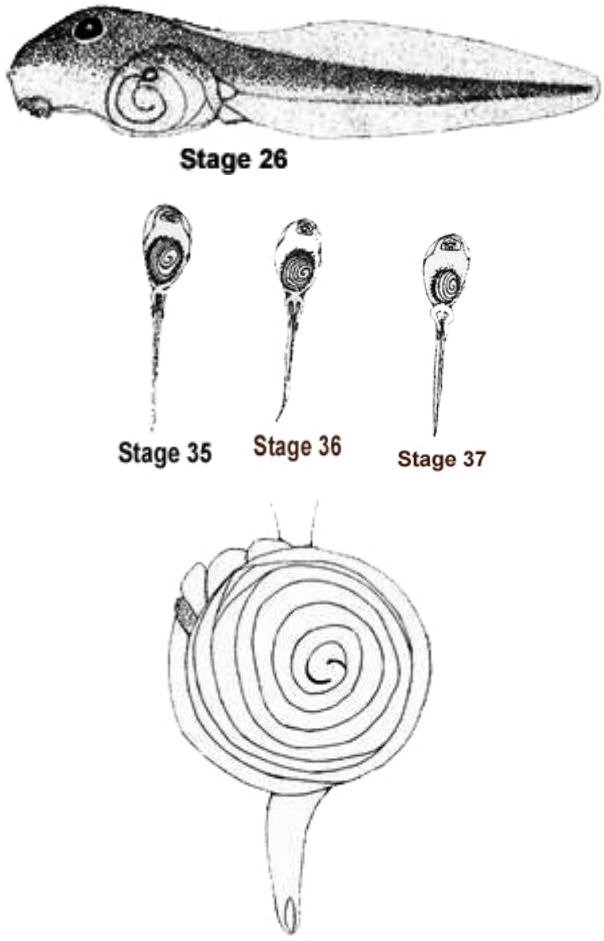


Fig. 8. Digestive system at Stages 26-37.
For abbreviations see Fig. 1.

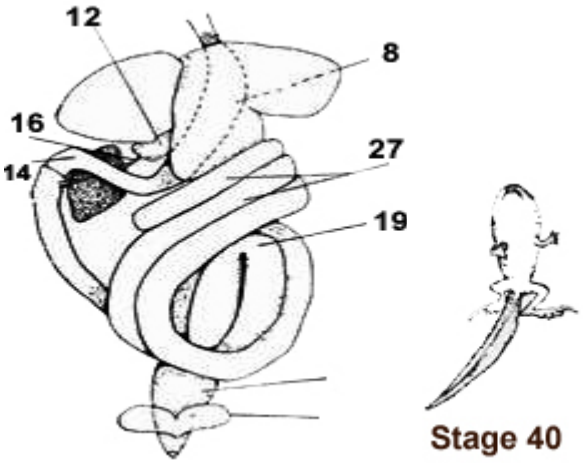


Fig. 9. Digestive system at Stages 38-40.
For abbreviations see Fig. 1.

Gastric region become thicker; liver lobes enlarged, bile duct runs obliquely downward to join pancreatic duct, hepato-pancreatic duct opens into duodenum. Vigorous feeding, expulsion of lumps of fecal matter is aided by vigorous tail movements.

Digestive system: Metamorphic changes in the gut become apparent: keratodonts are lost; intestinal coils reduced in number (Fig. 9): anti-clockwise spiral is reduced to 5,1/2, while clockwise to 4 coils; liver is enlarged, right lobe with two distinct large lobes, it hides stomach from ventral view; belly does not bulge; stomach is shortened and thickened due to disappearance of esophago-gastric and gastro-duodenal flexions; rectal part of large intestine becomes pear-shaped; length of gut reduced to 65-89 mm; bilobed urinary bladder is indicated (Fig. 9).

Stage 40, 41 (Fig. 10)

Stage criteria: Fore limbs break through the atrium; eyes characteristically protrude, with slight movements; tail reduced to 1/4th of its size; patch of skin extends on dorsum and lateral sides; head contracts becomes more massive.

Digestive system: Horny jaws are lost, mouth slit widens sideways; duodenum straightened up obliquely; coils represent ileum, digestive canal measures 18 mm in length (Fig. 10).

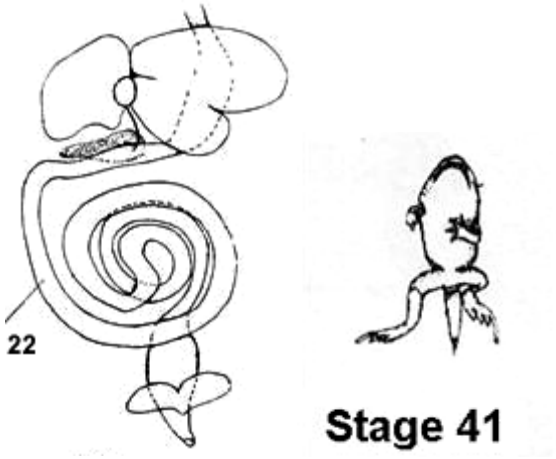


Fig. 10. Digestive system at Stage 40-41.
For abbreviations see Fig. 1.

Stage 42 (Fig. 11)

Stage criteria: Tail reduced to a black tipped stump; tuberculate skin covers the body; the toadlet hops out of water into surrounding vegetation, when disturbed it jumps back in water.

Digestive system: Stomach obliquely placed with distinct cardiac, and pyloric ends, coils disappear, ileum is straightened out; rectum widens, with distinct bilobed urinary bladder. The gut measures 13 mm in total length (Fig. 11).

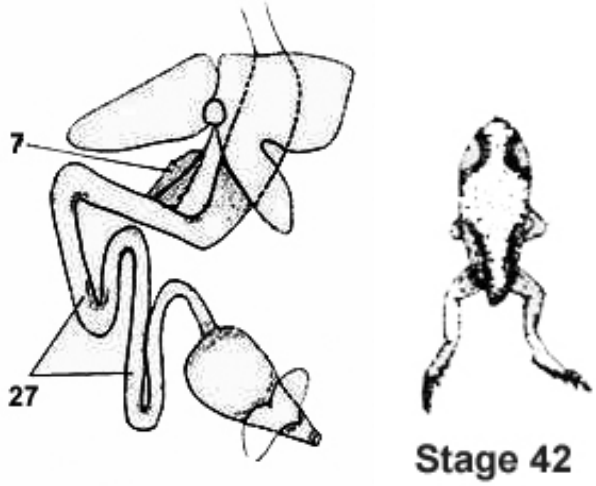


Fig. 11. Digestive system at Stage 42.
For abbreviations see Fig. 1.

Stage 43 (Figs. 12, 13)

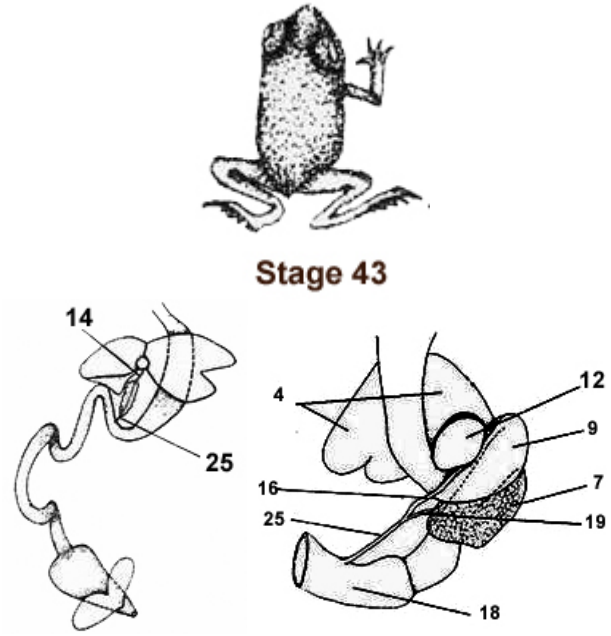
Stage criteria: Skin with warts; eyes

protruded with lids; limbs and digits are elongated; the toadlet hops about in the grass around the pond, feeding on small insects.

Digestive system: Mouth gape extends beyond the level of eyes. The gut consists of duodeno-iliac loop, central loop (ileum), and pear-shaped rectum. Cloacal aperture is round with sphincter muscles (Fig. 12), with distinct pancreas and hepato-pancreatic duct opening in the proximal end of the duodenum (Fig. 13).

Change in tadpole's behavior during development

At Stage 25, color of *Bufo stomaticus* tadpoles is sooty black. They form loose feeding-swarms along marginal water of ponds and puddles, a universal behaviour among bufonid tadpoles (Allee, 1931; Bragg, 1965;Wassersug, 1973). They are unpalatable to herons and other predators (Khan, 1965, 1991), However, after Stage 33 there is marked change in tadpole's behaviour. It becomes palatable, turns grey with dark spots, becomes solitary, as it turns palatable. It feeds vigorously, body becomes bulky, and is very agile, when disturbed, quickly retreats in the roots of water plants, or buries in the gravel at bottom. Similar ontogenetic behavioural changes have been reported in other *Bufo* species (Crump, 1984; Heyer *et al*. 1975).



Figs. 12 and 13. Digestive system at Stage 43. For abbreviations see Fig. 1.

DISCUSSION

As soon the neural tube is established (Stage 15), the embryo rapidly increases in size, aggregation of melanophores mark positions of proctodium and stomodium (Stage 21, Fig. 1). During next ten stages gut spirals and associated glands are developed (Figs. 5-7). Feeding starts at Stage 25, when tadpole measures 6.8-7 mm and gut 6-6.7 mm, with two and half ascending and one and half descending coils. Maxima is reached at Stage 37 ("critical stage": Khan, 1965), tadpole is 19-20 and gut measures 125 mm comprising 9 ascending and 8 descending coils. These values dwindle fast during metamorphosis, so 6.6-7 and 10-12 mm at toadlet stage, gut forms two loose loops (Table I, Fig. 14), oral sucker and tail are lost, fore limbs erupt, hind limbs become functional, switching over from aquatic to aerial respiration, herbivory to carnivory, swimming to saltation, head shrink, mouth gape widens, body wall thickens, skin is tuberculated, gut is differentiated into esophagus, stomach, duodenum, ileum, rectum, with associated glands: liver, pancreas and spleen and urinary bladder is developed (Figs. 9-13).

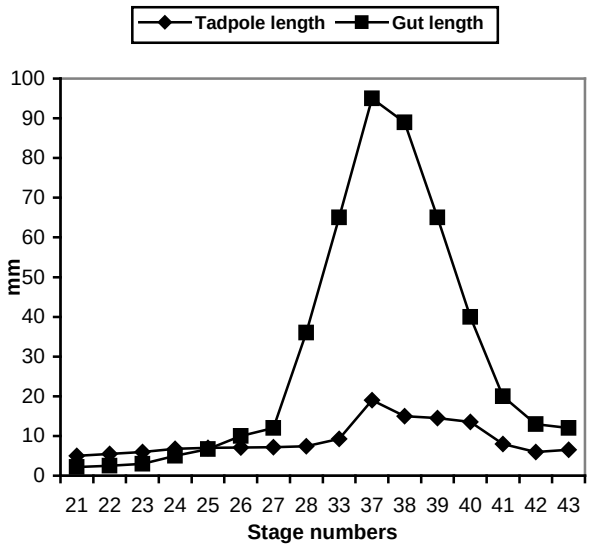


Fig. 14. Graphic presentation of variations

in body and gut size during developmental stages of *Bufo stomaticus*.

The intrinsic secretions "spemann organizer" control pre larval and larval organogenesis (Thibaudeau and Altig, 1988; Harland and Gerhart, 1997; Nieto, 1999), while thyroid hormone the metamorphosis. The digestive system remains functional from 21^{th} to 34^{th} Stage, however, at 35^{th} the upsurge of thyroid hormones in the blood stream changes the life style of the larva. It stops feeding as destructive and resorptional metamorphosic processes set in (Nakajima *et al*., 2005). Parallel has been observed in human prenatal development, at birth thyroid secretions surge in fetal blood stream changes serum protein to albumen, necessary to switch over to air as oxygen source, rather than maternal blood stream (Stubbe *et al*., 1978; Tata, 1993). Metamorphosis is the most dramatic phenomenon in vertebrate development, it remodels larval body plan into adult (Leloup and Buscaglia, 1977; Fox, 1981), involving programmed cell death (Nakajima *et al*., 2005) by non-lysosomal and lysosomal bodies (Clarke, 1990; Weber, 1964).

Table I.- Variations in gut length, number of ascending and descending of intestinal coils during development of *Bufo stomaticus*.

Stage No.	Tadpole length (mm)	No. of ascending coils	No. of descending coils	Gut length (mm)
21	4.6-5.0	-	-	2.2
22	5.3-5.5	-	-	2.5
23	5.5-6.00	-	-	3.0
24	6.0-6.8	1-1.5	1-1.5	4.5-5.0
25	6.8-7.0	2-2.5	1-1.5	6-6.7
26	7.1	3-3..5	2-3	9.5-10
27	6.95-7.2	3-3.5	2.5-3	9.5-12
28	7.0-7.4	3.5-4	3-3.5	25-36
33	8.9-9.3	4.5-5	3.5-4.5	50-65
37	18-19	7-9	7-8.5	95-125
38	14-15	5.5-7	4-6	65-89
39	13.5-14.5	3.5-4	2-3	52-65
40	12-13.5	1.5-2.5	2-3	32-40
41	7.0-8.0	1	1	18-20
42	5.2-6.0	-	-	13
43	6.5	-	-	10-12

Most of the metamorphosic biochemical studies have been done on *Xenopus laevis* tadpole, none so far on *Bufo stomaticus*. Table I in Khan (1965) compared developmental tables of both species. The biochemical activity occurring in metamorphosing *stomaticus* tadpole are inferred from data available for *X. laevis* at comparable *B. stomaticus* stages (in parenthesis). The intrinsic secretion period extends up to stage 54 (28); thyroid gland secreting Stages are 55-57 (32-35); the hormonal concentration is at climax 58-66 (38-43). Forelimbs erupt, head shrinks, gills and tail is resorbed, skin, intestine and pancreas are remodeled (Nishikawa and Hayashi, 1994; Dodd and Dodd, 1976). Cell death reaches peak level in skin at Stage 60 (38), intestine at 61 (40), and tail muscles at Stage 62 (43) (Khan, 1965; Ishizuya-Oka *et al*., 2000; Schreiber and Brown, 2003; Nakajima and Yaoita, 2003).

Comparatively cell death is rapid in intestine reducing it to 90% of its length (Marshall and Dixon, 1978). It is thickened, becomes multilayered, epithelium is folded, elaborate connective tissue and muscle layers appear (Bonneville, 1963; McAvoy and Dixon, 1977).

Different parts of the hind limb become functional and help in anchoring the tadpole when it feeds on the marginal vegetation, at Stage 35. When disturbed, instead of diving in deep water it mostly scurries among roots of the grasses growing in shallow marginal water. At this stage the gills have disappeared and pulmonary respiration takes over. Arial respirations is facilitated in shallow water as naris are exposed to air. The tadpole stays there till completion of metamorphosis, when it climbs out of water as a toadlet.

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