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COMPARATIVE MORPHOLOGY AND pH OF THE ALIMENTARY TRACT IN *Archachatina marginata* AND A*chatina achatina*

***[1]OKENIYI, F.A. AND [2]OSINOWO, O.A.**

[1]Department of Animal Science, Landmark University, P.M.B 1001, Omu-Aran, Kwara State, Nigeria. [2]Department of Animal Physiology, Federal University of Agriculture, P.M.B. 2240, Abeokuta, Nigeria. *Corresponding Author Email: funmilayookeniyi@gmail.com. Phone; +2347038395662 **https://dx.doi.org/10.4314/jard.v20i1.8**

ABSTRACT

A study was carried out to investigate the comparative morphology and pH of the gut in the Giant African Land Snails, in Archachatina marginata and Achatina achatina respectively. The crop is long, distended and less fleshy with reddish brown coloured juice (5.8 ml volume) in A. marginata, while it is short, compact and fleshy with greenish brown coloured juice (3.2 ml volume) in A. achatina. The digestive gland was heavier (17.36 vs. 15.74 g) in the latter than the former. The absolute weight of the crop in A. marginata was significantly (p<0.05) higher than in A. Achatina, however, the absolute and relative weights of the digestive gland in A. achatina was significantly (p<0.05) higher than in A. marginata. There were significant (p<0.05) differences in linear measurements of the salivary gland and crop for absolute values and the salivary gland, crop, stomach and digestive gland for relative values. Archachatina marginata had significantly (p<0.05) longer salivary gland and crop than Achatina achatina for absolute length values and significantly (p<0.05) longer salivary gland, crop and digestive gland for relative length values. However, the relative stomach length in A. achatina was significantly (p<0.05) longer than in A. marginata. The gut pH was more acidic in the crop (5.38 and 4.83) than in the mouth (6.93 and 6.82) where it tended towards neutrality in A. marginata and A. achatina respectively. The pH profiles in the two species, decreased by 1.65 and 1.99 from the mouth to the crop while it increased by 1.46 and 1.97 from the crop to the distal digestive gland in A. marginata and A. achatina respectively. In the herbivorous snails, the pH along the gut regions remained acidic all through (5.38 – 6.93 and 4.83 – 6.82) in A. marginata and A. achatina respectively, confirming the herbivorous habits in these snail species. The longer gut and distended crop with higher volume of crop juice in A. marginata, implies its ability to store, utilize and digest more food materials than A. achatina. These results provide the basis for a better understanding of the physiology of the digestive processes in these snails.

Keywords*: pH profile, Morphology, Gut length, Gut weight, A. marginata, A. achatina*

INTRODUCTION

Snail farming is fast gaining popularity all around the globe including the continent of Africa. Snail species are many with new species being discovered and others being rediscovered (Thach, 2015 and Schilthuizen, 2017). In West Africa, the commonly farmed snails are the giant African land snails. Captive rearing of the giant African land snails is profitable agri-business due to the low capital inputs and numerous benefits of the snails. Snails are known for their production of high-quality meat, health benefits, medicinal and cosmetic use of the haemolymph and mucin, use of the shell and offal, with profitable income from sale of snail meat and its products. In Nigeria, unlike what obtains for conventional and other mini livestock, there is yet to be any standard nutrient requirements to guide snail farmers in ration formulation for the giant African land snails.

To achieve this, many studies had been carried out using different feed materials to feed snails but no standard has been set. It is however imperative to first have a detailed understanding of the anatomy and physiology of digestion in the gastrointestinal tract of the giant African land snail.

The snail gut, like that of most animals, consists of an oesophagus leading from the mouth to the stomach followed by a long intestine ending in an anus (Martin *et al*., 2011). Therefore, as is generally found in stylommatophorans the digestion process starts in the mouth and ends in the anus. The snail gut regions are involved in food reception, storage, digestion, nutrient absorption and faeces formation (Dimitriadis, 2001). Earlier studies showed that food is gathered into the mouth by movement of the radula over the tip of the Odontophore and by movement of the Odontophore itself (Mackenstedt and Markel, 1987). The function of the salivary gland is believed to be lubrication, thus assisting with the removal of food from the radula and its passage into the oesophagus. The digestive gland or the hepatopancreas of gastropod molluscs is the key organ for metabolism. It is the main source of production of digestive enzymes, and it is involved in absorption of nutrients, food storage and excretion (Baker, 2002). Hepatopancreas is also implicated in storage and excretion of inorganic reserves, lipids and carbohydrate metabolites (Zarai*et al*., 2011). Intestine length is determined by diet type in some animals and as such they balance nutritional needs to meet energy requirements through phenotype adjustment (Karasov and Douglas, 2013). Determination of morphological characteristic for the exploration of the spatial and trophic niche (Pagotto *et al*., 2011) is necessary in identifying adaptive ecomorphological patterns due to selection. Giant land snails are well equipped with a wide range of digestive enzymes (Ademolu*et al*., 2013) with earlier studies showing that land snails often eat plants high in protein and calcium nutrients (Omole *et al*., 2011), and they also partake in leaf litter decomposition just like other invertebrates living on the soil (De Oliveira *et al.*, 2010).

As part of studies carried out to investigate the physiology of digestion in the giant African land snails, the morpho-anatomy and pH profile of alimentary tract in *A. marginata* and *A. achatina* were studied and compared. Little work of this kind has been carried out in the giant African land snails and in this study the gut anatomical structures and pH profile in the two species of giant African land snails were investigated.

MATERIALS AND METHODS

Experimental Animal

Adult snails (150-170 kg weight) of *A. marginata* species (20 snails) and A. *achatina* (20 snails) used in this study were obtained from local snail market. The snails were kept in clean, basket units of 45 x 20 x 21 cm, each containing 5 snails per basket for adequate spacing. Each snail was given adequate feed (Pawpaw leaves and fruits) and water (ad libbitum) and allowed to acclimate for 2 weeks prior to its use.

Morphoanatomy

Snails were aseptically dissected following the methods of Segun (1975) and identification of the gut regions was carried out. The organs of the alimentary tract were extracted to identify the regions of the gut, namely mouth, radula, buccal mass, anterior oesophagus, posterior oesophagus, crop, salivary gland, Stomach, small intestine, rectum, hepatopancreas and anus. Pictures of the overall view and pictures of individual digestive tract organ of the snail were taken using a digital camera.

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Morphometric Data Collection
The measurements of the organs of the alimentary tract extracted were taken, length and width of the organs were measured by means of meter ruler, cotton thread, and vernier calliper, while the weights of each of the parts was determined using a sensitive scale (Ohaus Adventurer - 0.001 g sensitivity).

Determination of pH
The measurements of the gut pH were carried out using pH meter. The digestive tract of *A. marginata* and *A. achatina* were excised and cut into the gut regions after dissection. Individual gut regions were homogenized with the gastro-intestinal tract contents and an equal volume of phosphate buffer in a Potter homogenizer (Potter and Elvehjem, 1936) which was fitted with a Perspex pestle and surrounded by ice. The homogenate pH of the snail gut was determined using the pH meter.

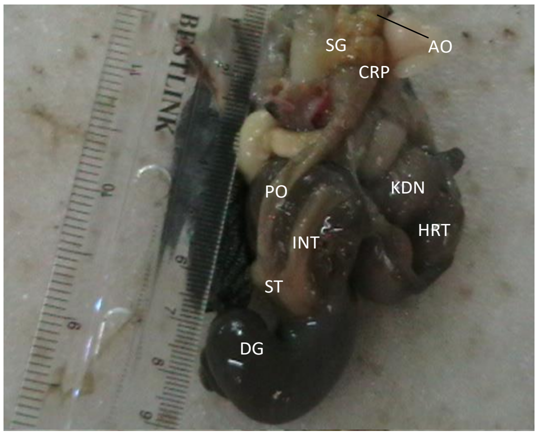
Statistical analysis
Morphometric data were subjected to the pooled variance 't' test (Systat Inc., 2004) for comparison between species. pH data were subjected to analysis of variance (ANOVA) using the Systat Analytical Computer Package version 5.02 (Systat Inc., 2004) in a 2 x 11 factorial arrangement (2 snail specie x 11 gut regions) in 3 replicates. Significant means were separated using Turkey's highest significant difference (HSD).

RESULTS

A general overview of the digestive tract (Plates 1A and 1B), a schematic diagram (Plate 2) representing the two species of GALS and specific views of individual digestive organs (Plate 3) showed that the digestive system of GALS consists of a mouth, a buccal mass, an anterior portion of the oesophagus, a pair of salivary glands with a pair of salivary ducts connecting the anterior portion of the gland to the dorsoposterior surface of the buccal mass, a crop, on the surface of which the pair of salivary glands are located, a posterior portion of the oesophagus, a stomach, an intestine, a digestive gland which connects to the stomach via a short duct and to the posterior portion of the oesophagus via a longer duct, and finally a rectum leading to an anus.

The Foregut: mouth, buccal mass, anterior oesophagus, crop and posterior oesophagus
It was observed that the mouth is located at the middle of the snail head and opens into the buccal mass. It is circular (anterior and ventral) when fully extended and surrounded by lips and a jaw which facilitates the extension of the snail's mouth when eating. The radula shape is a concave bend and inside the mouth on the anterior of the buccal mass. It is yellowish brown in *A. marginata* but reddish brown in *A. achatina*. It consists of rows of teeth which is sharp and scratchy to touch. The buccal mass (BM) follows immediately after, with its upper surface connected to the salivary glands by thin salivary ducts, and opens into the anterior oesophagus. The anterior oesophagus is a short tubular structure protruding from the posterior of the BM and on its posterior and ventral surfaces laid the elongated tubular salivary ducts linking the buccal cavity. The crop is thin walled, bulbous, highly distended and filled with reddish brown crop juice in *A. marginata* but in *A. achatina* the crop juice is dark greenish brown and the crop is less distended and thick walled. The mean volume of the crop juice (Figure 1) differs being 3.5ml and 5.8ml in *A. Achatina* and *A. marginata*. respectively. In *A. marginata* the crop is large and contains a higher crop juice volume compare to *A. achatina*.

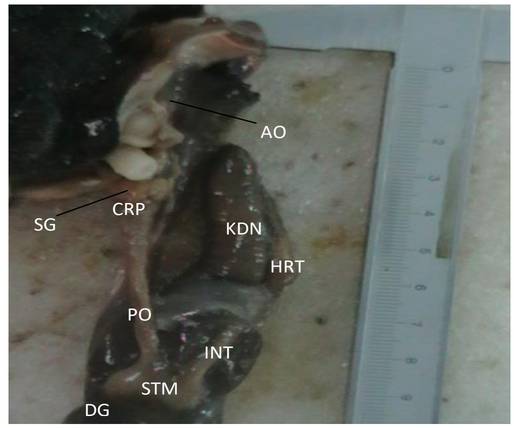
The crop, in the two snail species, connects the short anterior oesophagus to the long posterior oesophagus. The posterior oesophagus is a long tubular structure linking the posterior end of the crop to the stomach. It occupies about the middle of the snail body passing directly into the stomach, it connects to the digestive gland via digestive gland duct.



A. An overview of the digestive system of *A. marginata*

SG= Salivary gland, CRP= Crop, PO= posterior oesophagus, INT = Intestine, STM= stomach, KDN= Kidney, HRT= Heart, AO= Anterior Oesophagus, DG= Digestive gland.

Plate 1A: Overview of the Digestive System in *Archachatina marginata*



B. An overview of the digestive system of *A. achatina*

SG= Salivary gland, CRP= Crop, PO= posterior oesophagus, INT = Intestine, STM= stomach, KDN= Kidney, HRT= Heart, AO= Anterior Oesophagus, DG= Digestive gland.

Plate 1B: Overview of the Digestive System in *Achatina achatina*

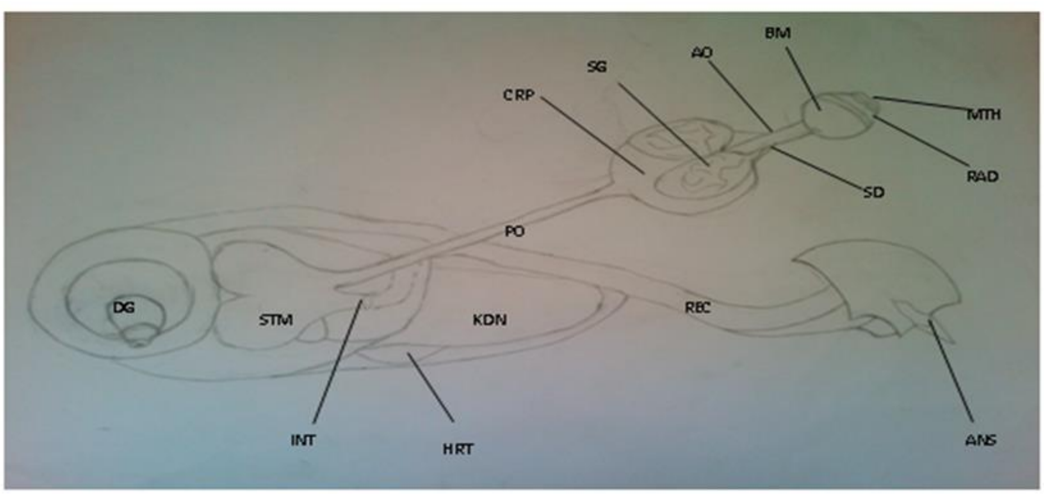


Plate 2: The schematic diagram of the alimentary tract of the Giant African Land Snails (GALS). Mouth = MTH, Radula = RAD, Buccal mass = BM, Anterior Oesophagus = AO, Crop covered with salivary gland = CRP, Paired salivary glands = SG, Salivary ducts = SD, Posterior Oesophagus = PO, Stomach = STM, Digestive gland = DG, Rectum = REC, Heart = HRT, Kidney= KDN, Anus = ANS. Scale= x0.25

Comparative Morphology and pH of *A. marginata* & *A. Achatina* Gut

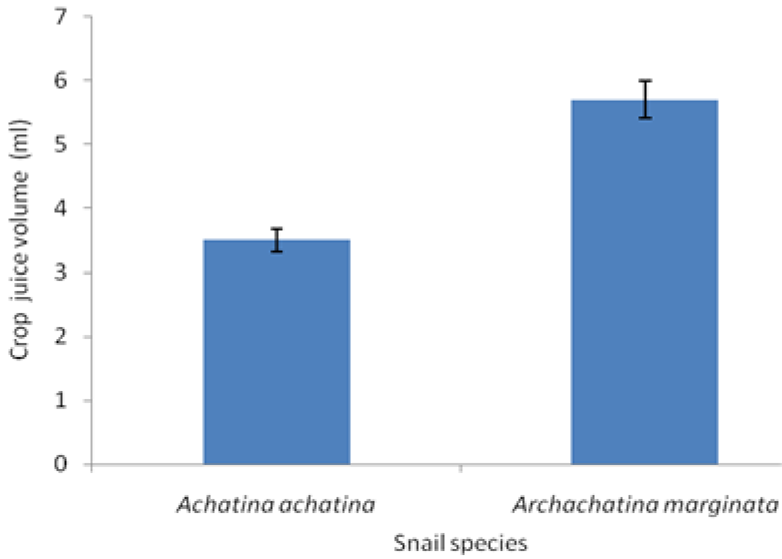


Figure 1: Crop juice volume in *Archachatinamarginata and Achatina achatina*

The Midgut: stomach and intestine
The stomach is a muscular, bi-lobed and u-shaped structure located in the posterior of the snail body. It is slightly embedded on the anterior portion of the digestive gland, facing the kidney and links both the posterior oesophagus anteriorly and the intestine posteriorly. The stomach connects to the digestive gland via a very short digestive gland duct in the mid portion of the gastric sac. The intestine is a thin long tube elongating from the stomach to the rectum. The intestine follows a path toward the left side of the stomach penetrating the digestive gland to form a c-shaped boundary between the digestive gland and the kidney before linking the thick cartilaginous rectum. The intestine is deeply embedded in the tissues of the digestive gland in *A. marginata*while the dept is shallower in *A. achatina*.

The Hindgut: rectum and anus
The rectum is located in the right mid-section of the snail's body cavity. The diameter is wider than the intestine and continues to widen slightly as it moves to open into the anus. The haepatopancreas is linked by a short duct to the stomach and a longer duct to the posterior oesophagus. It is the largest organ of the digestive system. It forms spiral curves that is widest at the anterior end and thins at the posterior end. The anus is located at the right mid ventral part of the snail body. The rectum opens into the anus and it contain an anal sphincter and a wide anal opening.

The Appendages: salivary and digestive glands
The salivary glands are a flat paired structure consisting of lobes. It is leaf-shaped and irregular, yellowish and with thick lobes. The salivary glands laced the entire surface of the crop; covering the ventral and dorsal surfaces and sides of the crop. The entire lobed structure is connected by short tubes linking the entire gland structure and also penetrating the crop. The salivary gland is also linked to the buccal mass by the elongated salivary ducts. The digestive gland / haepatopancrease is linked by a short duct to the stomach and a longer duct to the posterior oesophagus. It is the largest organ of the digestive system and even the entire snail body. It forms

spiral curves that is widest at the anterior end and thins at the posterior end. The summary of the features of the digestive system in *A. marginata* and *A. achatina* are as outlined in Table 1.

Morphometry

The absolute and relative weights of the different digestive tract organ of the GALS are presented in Table 2. There were similarities (p>0.05) in the weights of the different organs of the snail species, except that of the buccal mass, crop (absolute weight) and digestive gland, which showed differences (p<0.05) in weights. The absolute weight of the crop in *A. marginata* was higher (p<0.05) than in *A. achatina*, while the absolute and relative weights of the digestive gland in *A. achatina* was higher (p<0.05) than in *A. marginata*.

Tables 3 showed linear measurements (absolute and relative linear measurements, respectively) of the snail digestive tract organs. There were differences (p<0.05) in linear measurements of the salivary gland and crop for absolute values and the salivary gland, crop, stomach and digestive gland for relative values. *A. marginata* had longer (p<0.05) salivary gland and crop than *A. achatina* for absolute length values and longer (p<0.05) salivary gland, crop and digestive gland for relative length values. However, the relative stomach length in *A. achatina* was longer (P<0.05) than in *A. marginata*. The lengths (absolute and relative) of all other organs of the digestive tract were not affected (p>0.05) by species differences of GALS.

Table 4 shows the mean values of width measurements of the organs of the snail digestive tract. There were no differences (p>0.05) between snail species in width of organs of the digestive tract, with the exception of the buccal mass and crop. *A. marginata* had higher (p<0.05) width than *A. achatina*. The width of all other organs of the digestive tract were not affected (p>0.05) by gut region in both species of GALS.

Table 2: Mean Absolute (g) and Relative Weights (%) of Digestive Tract Organ of A*rchachartina marginata* (AM) and *Achatina achatina* (AA)

	Absolute weight (g)		Relative weight (%)	
Gut region	**AA**	**AM**	**AM**	**AA**
Mouth	0.33 ± 0.06	0.66 ± 0.29	0.14 ± 0.03	0.40 ± 0.17
Radula	0.02 ± 0.00	0.02 ± 0.01	0.01 ± 0.00	0.01 ± 0.00
Buccal mass	1.22 ± 0.25 [a]	0.71 ± 0.06 [b]	0.52 ± 0.09	0.43 ± 0.03
Anterior oesophagus	0.13 ± 0.02	0.10 ± 0.03	0.05 ± 0.01	0.06 ± 0.02
Salivary gland	0.64 ± 0.14	0.45 ± 0.02	0.27 ± 0.05	0.27 ± 0.01
Crop	1.44 ± 0.39[a]	0.69 ± 0.04[b]	0.62 ± 0.18	0.42 ± 0.03
Posterior oesophagus	0.59 ± 0.30	0.32 ± 0.06	0.25 ± 0.12	0.19 ± 0.03
Stomach	1.30 ± 0.22	1.18 ± 0.30	0.56 ± 0.11	0.71 ± 0.19
Intestine	0.40 ± 0.02	0.34 ± 0.11	0.17 ± 0.01	0.21 ± 0.065
Digestive gland	15.74 ± 0.02 [b]	17.36 ± 2.11 [a]	6.73 ± 0.25[b]	10.46 ± 1.43 [a]
Rectum	2.78 ± 0.49	2.06 ± 0.72	1.19 ± 0.20	1.25 ± 0.45
Anus	2.10 ± 0.83	1.7 ± 0.72	0.90 ± 0.38	1.05 ± 0.16
Whole gut	31.32 ± 1.19	25.59 ± 1.74	13.39 ± 0.95	15.43 ±0.84
Live weight	233.89 ± 5.18 [a]	166.23 ± 2.57 [b]		

[a, b,]Means with different superscripts in the same row differ significantly (p<0.05) for absolute and relative weights independently. Values are given as mean ± SEM

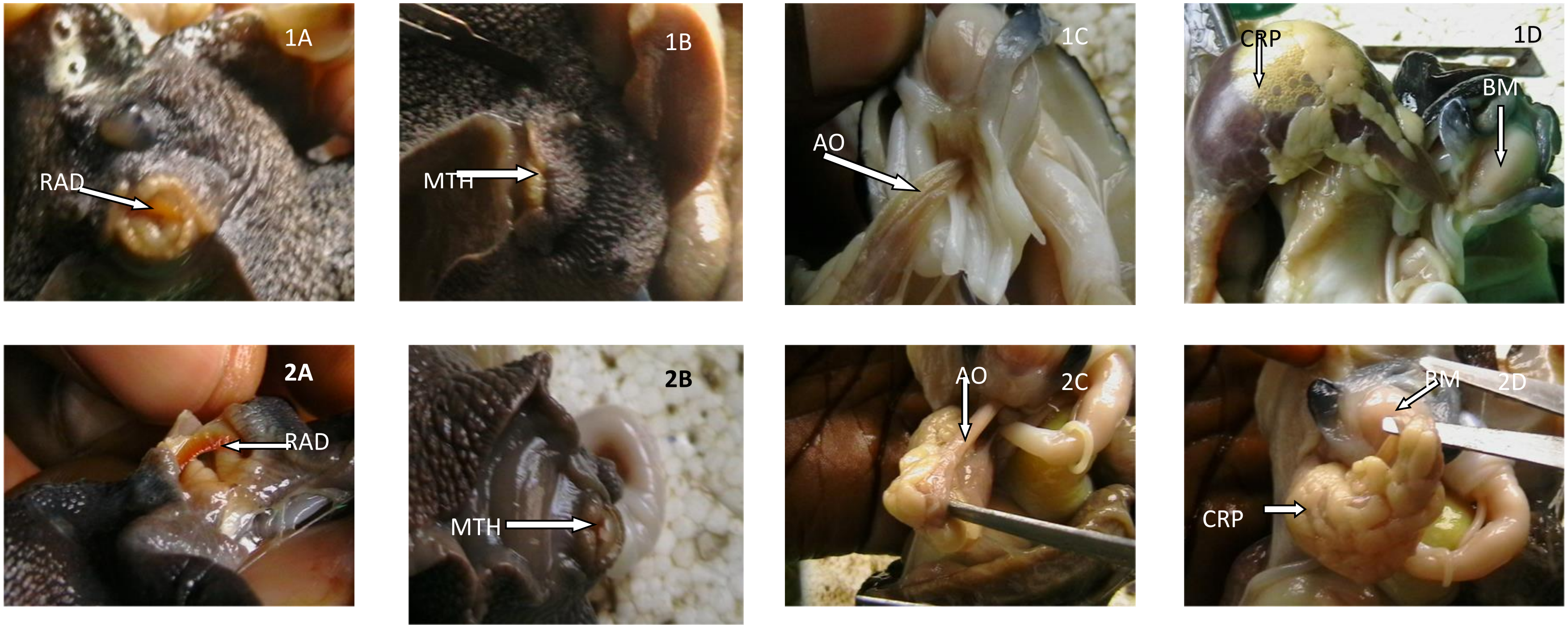


Plate 3: The Organs of the Alimentary Tract in *A. marginata* (1 A-D) and *A. achatina* (2 A-D).
Radula = RAD, Mouth = M, Buccal mass = BM, Anterior Oesophagus = AO, Crop covered with salivary gland = CRP, Paired salivary glands = SG, Posterior Oesophagus = PO, A-D: Scale= x0.25

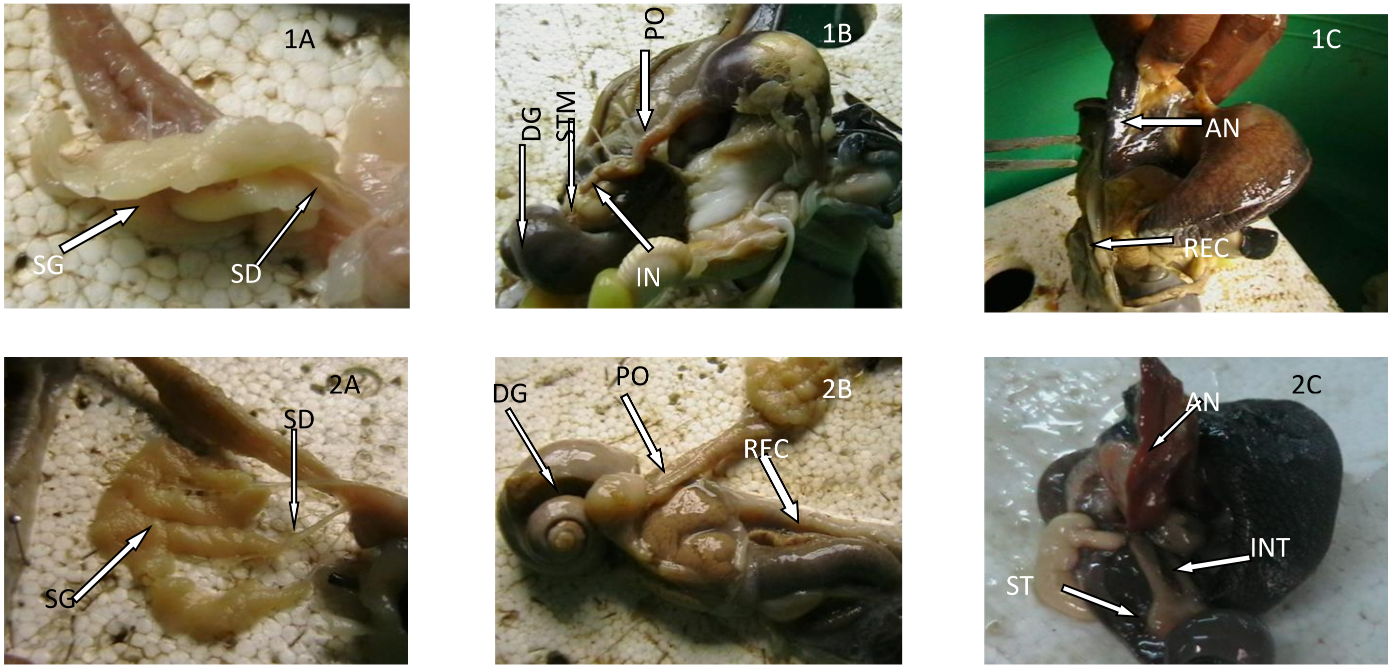


Plate 4: **The Organs of the Alimentary Tract in *A. marginata* (1A-C) and *A. achatina* (2A-C)**.
Paired salivary glands = SG, Paired salivary ducts = SD, Posterior Oesophagus = PO, Stomach = STM, Intestine = INT, Hepatopancreas/Digestive gland = DG, Rectum = REC, Anus = ANS. A-C: Scale= x0.25

Comparative Morphology and pH of *A. marginata* & *A. Achatina* Gut

Table 1: Summary of the Features of the Digestive System of *A. marginata* (AM) and *A. achatina* (AA)

Organ	Position in snail body	Colour and shape
Mouth	Anteriorly ventral on the head with lips and radula	Dark brown sometimes with a patch of light brown in AM. Circular when fully Protruded.
Radula	Inside the mouth and anteriorly on the BM	Concave shape. Yellowish brown in AM while Reddish brown in AA
Buccal mass (BM)	Anterior body portion.	Cream and Pear -shaped
Anterior Oesophagus	Arises from dorso posterior surface of the buccal mass. It is short with the salivary duct wrapped round it	Light brown, Elongated and tubular
Crop	Posterior body part, between anterior and posterior oesophagus. It is slightly distended and thick walled in AM but thin walled in AA	Spherical, Reddish brown when filled with crop juice in AM while Brown even when filled with crop juice
Posterior oesophagus	Dorsal mid-body section. It is a long tube linking the crop to the stomach	Light brown Elongated and tubular
Stomach	Posterior body portion and embedded on the surface of the anterior portion of the DG. It is tough, thick and muscular	Cream Bi-lobed and thick walled
intestine	Anterior portion is embedded in DG (slightly in AM but deeply in AA), forms a c-shaped curve along the boundary between the kidney and DG. It is thin walled and long	Cream, elongated and tube
Digestive gland (DG)	Dorsal body portion. It contains tubules and connecting ducts linked to the posterior oesophagus and stomach.	Spiral shape. Dark in AM while Dark brown in AA
Salivary glands	Covers the entire width and ¾ length of the bulbous crop. Contains several lobes connected by thin ducts to the buccal mass and crop.	Thin, irregular, flat and leaf-shaped. Cream in AM while Yellow in AA
Rectum	Right mid body section, with a wider diameter than the intestine	White, elongated and thick cartilaginous tube
Anus	Right middle ventral body section. The opening is wide	Slightly curved. Dark in AM while Reddish brown in AA

Table 3: Mean absolute (cm) and relative linear measurements (%) of digestive tract organ of *Archachartina marginata*(AM) and *Achatina achatina* (AA)

	Absolute measurements (cm)		Relative measurements (%)	
Gut region	**AA**	**AM**	**AM**	**AA**
Mouth	0.58 ± 0.45	0.41 ± 0.25	2.08 ± 1.34	1.85 ± 1.08
Radula	0.90 ± 0.10	0.60 ± 0.44	3.44 ± 0.77	2.65 ± 1.89
Buccal mass	1.13 ± 0.31	0.85 ± 0.15	4.21 ± 0.75	3.84 ± 0.82
Anterior oesophagus	0.80 ± 0.00	0.85 ± 0.25	3.03 ± 0.36	3.85 ± 1.28
Salivary gland	5.60 ± 1.00 [a]	2.50 ± 0.20[b]	20.94 ± 1.47 [a]	11.27 ± 1.33[b]
Crop	5.35 ± 0.75 [a]	2.70 ± 0.36[b]	20.07 ± 0.79 [a]	12.18 ± 2.01[b]
Posterior oesophagus	3.50 ± 0.40	3.18 ± 0.48	13.15 ± 0.41	14.32 ± 2.15
Stomach	1.10 ± 0.10	1.15 ± 0.13	4.14 ± 0.16[b]	5.16 ± 0.39 [a]
Intestine	5.60 ± 0.50	5.08 ± 1.03	21.09 ± 0.86	22.89 ± 4.68
Digestive gland	14.30 ± 1.70	10.38 ± 2.09	54.72 ± 12.71[a]	46.72 ± 9.29[b]
Rectum	7.02 ± 1.37	7.28 ± 1.79	26.21 ± 2.05	32.65 ± 7.22
Anus	0.65 ± 0.35	0.42 ± 0.20	2.57 ± 1.60	1.86 ± 0.85
Whole gut length	26.63 ± 3.30 [a]	22.25 ± 0.91[b]		

[a,b,] Means with different superscripts in the same row differ significantly ($P<0.05$) for absolute and relative linear measurements independently. Values are given as mean ± SEM

Table 4: Mean Width (cm) of Digestive tract organs of *A. marginata* and *A. achatina*

Gut region	AM	AA
Mouth	0.95 ± 0.21	0.58 ± 0.45
Radula	0.10 ± 0.00	0.23 ± 0.23
Buccal mass	1.00 ± 0.10 [a]	0.70 ± 0.10[b]
Anterior oesophagus	0.20 ± 0.00	0.20 ± 0.17
Salivary gland	4.40 ± 0.90	1. 80± 0.00
Crop	1.75 ± 0.15 [a]	0.80 ± 0.10[b]
Posterior oesophagus	0.20 ± 0.00	0.13 ± 0.06
Stomach	1.10 ± 0.10	1.05 ± 0.13
Intestine	0.17 ± 0.05	0.17 ± 0.06
Digestive gland	2.67 ± 0.12	2.55 ± 0.22
Rectum	0.40 ± 0.10	0.43 ± 0.06
Anus	2.10 ± 0.20	1.87 ± 0.25

[a, b] Means with different superscripts in the same row differ significantly ($P<0.05$) Values are given as mean ± SEM

pH of the Alimentary Tract in GALS

The pH measurement of the different regions of the digestive tract of GALS is shown in Table 5 and Table 6. The highest pH level was recorded in the mouth, foregut region, (6.93 and 6.82 for *A. marginata* and *A. achatina* respectively). *A. marginata* mouth is however less acidic than *A. Achatina* mouth, with the mouth pH tending towards neutrality in both species. The most acidic region of the foregut is the salivary gland in *A. Achatina* while it is the buccal mass in *A. marginata*. The lowest pH was found in the crop for the mid gut (4.83 and 5.38 for *A. achatina* and *A. marginate* respectively). *A. achatina* crop juice is more acidic than *A. marginata* crop juice. pH was higher ($p<0.05$) for *A. marginata* than *A. achatina* in all regions of the mid gut. Within both species anterior oesophagus had a higher ($p<0.05$) pH than other regions of the mid gut except the crop.

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The pH profiles in the two species, decreased by 1.65 and 1.99 from the mouth to the crop while it increased by 1.46 and 1.97 from the crop to the digestive gland in *A. marginata* and *A. achatina* respectively. The pH along the gut regions remained acidic all through (5.38 – 6.93 and 4.83 – 6.82) in *A. marginata* and *A. achatina* respectively. There was a higher ($p<0.05$) intestinal pH in *A. marginata* than in *A. achatina*. In both species digestive gland had a higher ($p<0.05$) pH than other regions of the hindgut. The pH measurements of the whole gut for the two species of GALS (*A. marginata* and *A. achatina*) were different ($p<0.05$) from each other, gut pH for *A. marginata* species, was higher ($p<0.05$) than that of *A. achatina*. However, on the basis of gut region division there were no differences ($p>0.05$) in the pH values of *A. marginata* and *A. achatina* species based on gut regions i.e., the pH of the fore- mid- and hind-gut regions were not significantly different.

Table 5: Effect of Snail Species and Gut Region on pH Profile of the Alimentary Tract of GALS

Gut region	AM	AA
Anterior/Foregut		
Mouth	6.93 ± 0.02^{a}	6.82 ± 0.00^{b}
Buccal mass	6.34 ± 0.02	6.46 ± 0.01
Anterior oesophagus	6.67 ± 0.01^{a}	6.24 ± 0.22^{b}
Crop	6.51 ± 0.18^{a}	5.84 ± 0.12^{b}
Crop juice	5.38 ± 0.17^{a}	4.83 ± 0.08^{b}
Posterior oesophagus	6.00 ± 0.19^{a}	5.85 ± 0.01^{b}
Middle/Midgut		
Stomach	6.03 ± 0.28^{a}	5.98 ± 0.10^{b}
Intestine	6.75 ± 0.23^{a}	6.16 ± 0.04^{b}
Posterior/Hindgut		
Rectum	6.53 ± 0.04^{a}	6.54 ± 0.17^{b}
Appendages		
Salivary gland	6.53 ± 0.04^{a}	6.23 ± 0.08^{b}
Digestive gland	6.84 ± 0.09	6.80 ± 0.10

Table 6: Effect of Snail Species on pH Profile of the Major Parts of the Alimentary Tract of GALS

Gut region	AM	AA
Whole gut	6.41 ± 0.09^{a}	6.16 ± 0.10^{b}
Anterior/Foregut	6.60 ± 0.095	6.50 ± 0.095
Middle/Midgut	6.12 ± 0.095	5.75 ± 0.095
Posterior/Hindgut	6.70 ± 0.095	6.50 ± 0.095

DISCUSSION

In this study, the structural division of the snail's digestive tract into three major sections namely: foregut, midgut and hindgut, were similar to those reported in other studies (Nation, 2004; Lobo-da-Cunha et al., 2010a and 2010b; Martinez-Pereira*et al* 2013; De Oliveira and Cônsoli, 2020). The salivary and digestive glands are considered to be appendages of the snail digestive tract in agreement with earlier authors (Lobo-da-Cunha et al., 2010a and 2010b). The mouth in the two species is round, extensible thick lipped organ for picking up food materials; Authors have described snail mouth as crescentic slit (Sreenivasan, 1995) or a round opening Segun (1975). In this study it was observed that the radula helps GALS to hold, tear and cut food materials to smaller pieces corroborating the findings of Hanson (2020) that the radula can be used to cut food of larger size, however carnivorous species use it on their prey (Kantor and Puillandre, 2012). The number of the row of teeth were not ascertained in this study, it was however reported in earlier studies that the number of teeth in one transverse row as well as the number of rows is variable based on snail species Rumi *et al*. (2017), and Krings *et al.* (2019). In the two species of study, buccal mass is structurally similar in form and size and it has a number of protractor and retractor muscles which helps to move the radula, thereby enhancing the mechanism of feeding. An earlier study (Neustadter *et al*., 2007) revealed that in species like *Aplysia californica*, the buccal mass is suspended within the head by thin extrinsic muscles that facilitate rotation of the entire buccal mass during feeding thereby combining protraction and retraction with opening and closing in order to carry out the action of biting, swallowing and rejection.

The structure of the anterior oesophagus in GALS is similar to that of other snails possessing this structure. Sreenivasan (1995) reported that the anterior oesophagus is a short, thick-walled tube found in most gastropods. The crop seems to be the largest part of the foregut which stores and initially digests food materials. The larger and more distended structure of the crop with a higher crop juice volume in *A. marginata* species compared to *A. achatina* may be an adaptation for uncertain food supply, and when the food is available, a larger amount can be consumed and effectively digested per time. Charrier and Brune (2003) described the crop in terrestrial snails as enlarged, ectodermal sac surrounded by a pair of salivary glands. The posterior oesophagus tube opens unto the stomach, functioning to conduct and digest food materials in the two species of GALS. The u-shaped, thick-walled, muscular and bi-lobed stomach in both species of GALS is similar to earlier reports describing the stomach as thick-walled and muscular in euthyneuran mollusks, cylindrical in Cymbulia, bilobed in Lanx (Ghose, 1963), two plates in Onchidella (Fretter, 1943), and globular projections in Limnaea (Carriker, 1946). This study revealed that the intestine lies deeply imbedded in the anterior portion of the digestive gland in *A. marginata* but slightly imbedded in the anterior portion of the digestive gland in *A. achatina* in contrast to reports by Ghose, (1963) that the intestine is fully embedded in the digestive gland in *A. achatina*. In this study the tube-like rectum is thick walled in both species of GALS contrasting the report of Ghose, (1963) that the rectum is thin walled in *A. achatina.* The rectum links the exterior through the anus which opens beside the pneumostone, just behind the head in GALS as was also by Segun, (1975).

In this study the anterior lobe of the digestive gland opens into the crop and stomach in *Archachatina* and *Achatina* species via two digestive gland ducts. The longer duct opens into the posterior oesophagus linking the crop while the shorter duct opens into the stomach. This study corroborates the report of Ghose (1961), Charrier (1990) and Dar *et al*. (2017), that in land snail's digestive gland ducts open into two different sections of the gut, one opens in the stomach and the other in the crop. The discharge of the secretion of the digestive gland in the crop possibly accelerates the process of digestion, since the food is partly digested before it reaches the stomach. Also, saliva is mixed with the food in the buccal cavity, so digestion actually starts from there as reported by Dar *et al*. (2017). The digestive gland is heavier in *A. achatina* than in *A. marginata*. The reason for the heavier but shorter digestive gland in *A. achatina* is not well understood. However, the less heavy but longer digestive gland in *A. marginata* is indicative of its capacity for production of more crop juice, digestion and absorption of food materials and nutrients. The digestive gland in snails is very large and its secretion is conveyed to the stomach either by one or more digestive ducts, where the final stages of digestion take place (Carriker, 1946; Creek, 1953; Ghose, 1963; Lobo-da-Cunha, 2000). The digestive gland is involved in the extracellular and intracellular digestion of food. It serves other functions such as storage of lipids, glycogen and minerals; it is also the main site of nutrient absorption and plays a major role in detoxification (Nelson and Morton, 1979; Morton, 1983; Beeby and Richmond, 1988; Henry *et al*., 1991). Moreover, in some animal species it also stores secondary metabolites sequestrated from algae, which may be involved in the chemical defense system of these animals (Pennings, 1994).

Gut Morphometric

The gut represents the functional link between foraging (energy intake) and the energy available for survival, growth, and reproduction (Karasov, 1990; Secor, 2001). In this study, in terms of organ weight and length, the digestive gland had a higher value for both species of snails with *A. achatina* having a significantly heavier weight but shorter length than *A. marginata* in absolute and relative measures. The longer gut length of *A. marginata* may be indicative of the snail's ability to adapt to seasonal fluctuations in quantity and quality of food supplies. Earlier report showed that there is a strong relationship between intestinal tube length and diets in fish, such that they balance nutritional requirements against energetic cost by adjusting their phenotype (Wagner *et al*., 2009). Also, many animal species change their gut size seasonally as a consequence of fluctuating resource quantity and quality (Stark, 1999; Naya *et al*., 2009) for example, Eurasian perch developed longer guts when exposed to poorer quality food types both in the lab and in the wild (Olsson *et al*., 2007). Animals cannot maintain a unique digestive system that is simultaneously adapted for every type of diet because different food types are absorbed through different biochemical pathways and have different processing times (Karasov and Martinez, 2007; Karasov *et al*., 2011). Organisms need longer guts to digest lower quality food (e.g., fiber-rich) than higher quality food (e.g., protein-rich) (German and Horn, 2006; Wagner *et al*., 2009). Buddington *et al.* (1997) proposed that herbivores may have adapted to having absorptive tissues distributed along long, thin intestinal tubes, in order to enhance diffusion and absorption. The digesta would encounter more gut surface and spend less time at each position in the gut thus maintaining a higher diffusion gradient between nutrients in the bolus and those being absorbed across the mucosa. Longer gut tube may ensure that membrane-bound transport molecules operate at maximal rates when their target molecules are available in high concentration. In addition, longer guts have higher surface area and allow a longer retention time of the food, consequently enhancing nutrient absorption (Sibly, 1981). The wider and longer crop of *A. marginata* also suggests the ability to retain more crop juice produced by the digestive gland thus enhancing the digestive process within the snail.

Gut pH

Results of the present study showed that for the two species of herbivorous snails, the pH of the crop was the most acidic while the mouth pH is the least acidic, in the mouth the pH tended towards neutrality in both species. In general, *A. achatina* had a more acidic alimentary tract than *A. marginata* except at the buccal mass region of the gut. The pH profile gradient in the two species, decreased from the mouth to the crop but there after rises up unto to the distal digestive gland. In the herbivorous snails, the pH along the gut regions remained acidic all through, which may be due to the feeding habits of the snails and the type of gut micro flora present. The gut pH has implications on the bacteria flora type but not on the bacterial population within the gut microbial community (O'May *et al*., 2005; Dar *et al*., 2017). Oyeleke *et al.* (2012) reported that bacteria can also change the pH of the gut through fermentative reactions end products that affect the acid-base balance of the digestive tract. This study agrees with an earlier report (Walker *et al*., 1996; Charrier *et al*., 2006), that the pH of the crop is more acidic than the pH of the digestive gland. The lower crop pH may be attributed to the secretion of gut digestive enzymes and presence of food materials in the crop. Charrier and Brune (2003) documented a lower pH in the crop than in the distal intestine in gut pH of helicid snails. The gut of both snail species revealed an increase in pH along the gastrointestinal tract as found in reports of Charrier and Brune (2003).

CONCLUSION

The physiology of the digestive tract in the giant land snail is such that food material passes through the crop to the stomach while fine particles and soluble materials pass into the digestive gland; no food passes into the salivary glands, therefore the changes in pH that occurred in these organs were consistent with an effect of food material. The gastrointestinal tract is structurally similar in the two species of snail, starting in the mouth and ending in an anus. The longer and more distended crop in *A. marginata,* compared to *A. achatina,* implies its ability to, ingest, store, digest and utilize more food materials than *A. achatina.* These results provide the basis for a better understanding of the physiology of the digestive processes in these snails.

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