POLISH JOURNAL OF NATURAL SCIENCES
Abbrev.: Pol. J. Natur. Sc., Vol 39(4): 203–215, Y. 2024

DOI: 10.31648/pjns.9512

EFFECT OF LIVEWEIGHT ON HAEMOLYMPH BIOCHEMICAL AND HORMONAL PROFILE OF GIANT AFRICAN LAND SNAIL (*ARCHACHATINA MARGINATA*) UNDER TROPICAL ENVIRONMENT

***Oluwatosin Olawanle Ajiboye*[1], *John Adesanya Abiona*[2], *Oyegunle Emmanuel Oke*[3], *Okanlawon Muhammed Onagbesan*[4]**

[1] ORCID: 0000-0001-6610-2382
[2] ORCID: 0000-0002-1159-8349
[3] ORCID: 0000-0002-9425-4217
[4] ORCID: 0000-0002-9019-8828
[1–4] Department of Animal Physiology, College of Animal Science & Livestock Production Federal University of Agriculture, Abeokuta, Nigeria

Key words: liveweight, biochemical parameters, *Archachatina marginata*, Giant African Land, snail, hormonal profile of haemolymph.

Abstract

Demand for giant African Land snail (*Archachatina marginata*) is on the increase in recent years, but not much is known about the health indicators of this animal under indoor or intensive rearing. The aim of the study was to determine the effect of liveweight on biochemical and physiological parameters of *Archachatina marginata* and to establish reference ranges for these parameters. Snails used were assigned to 3 groups of 20 snails each based on their liveweight. Haemolymph collected was analyzed for biochemical, hormonal and selected minerals. Result showed that the effect of liveweight was not significant ($P > 0.05$) for Total protein (TP), Triglyceride (Trig), Alanine transaminase (ALT) and Glucose (Glu). However, Liveweight had significant effect ($p < 0.05$) on Albumin (Alb), Aspartate transaminase (AST), Cholesterol (Chl) and Creatinine (Crt).

For hormonal profile, only T_4 was significantly influenced ($p < 0.05$) by liveweight, while effect of liveweight was not significant on Triiodothyronine (T_3), Follicle-stimulating hormone (FSH), Estradiol (E_2) and Progesterone (P_4). For haemolymph minerals, liveweight only had significant effect on Cu and Fe among others. Physiological profiles quantitatively recorded for each of liveweight groups by the the present standard protocols provide a reference basis to quantify physiological changes under different environmental conditions.

Address: John Adesanya Abiona, Federal University of Agriculture, Abeokuta, Nigeria, e-mail: abionaja@funaab.edu.ng

Introduction

Blood analysis has become imperative for a number of reasons. One of such is for the health analysis of an animal. Blood samples are routinely used in disease surveillance and diagnosis in companion animal, livestock and human populations, at times providing the first indication of health abnormalities (WILLARD et al. 1994, FELDMAN et al. 2000, STOCKHAM and SCOTT 2002). The haemolymph of several marine bivalve species is useful in the identification of pathogens and the evaluation of mussel condition (PAILLARD et al. 1996, ALLAM et al. 2000). The analysis of blood constituents has been used to evaluate physiological stress in numerous marine and terrestrial animals including fishes (MEKA and MCCORMICK 2005), crustaceans (UHLMANN et al. 2009), snails (RENWRANTZ and SPILVOGEL 2011), and freshwater crayfish (MALEV et al. 2010). Similar assessment strategies based on the non-lethal collection of haemolymph of freshwater bivalves have been used in study to conserve and protect endangered and declining populations (GUSTAFON et al. 2005a).

By using giant African land snails (GALS), several studies were carried out on their reproductive performance (ADIO 2010, ABIONA et al. 2012, ABIONA et al. 2016, ABIONA et al. 2018, ABIONA et al. 2021), nervous system structure (Adebayo 2011), and circulatory system structure (Salami 2010). Comparative studies have been done on the haemolymph of *A. marginata* and *A. achatina* (SODIPE 2011, ABIONA et al. 2013, ABIONA et al. 2014) and on the duration of aestivation on biochemical and biophysical parameters of *A. marginata* (ABDUSAMMAD 2010). All of these published works were conducted by lethal sample collection methods.

By means of lethal blood sampling, haemolymph was collected to study the effect of parasites, including trematodes and nematodes relevant to human health, on their snail hosts (BROCKELMAN 1975, 1978, BROCKELMAN and SITHITHAVORN 1980) or to investigate compounds of biomedical importance e.g. c-reactive protein (CRP) (AGRAWAL et al. 1990). Veterinary surgeons and others investigating disease in captive snails may also need to take blood samples as part of diagnosis (COOPER and KNOWLER 1991). However, lethal sampling of a small number of animals is unlikely to reveal statistically convincing health information (WOBESER 1994, DORAN et al. 2001).

The assessment strategies based on the nonlethal repeated collection of haemolymph from GALS need to be developed for physiological examination of these animals. GALS has been identified as a health risk in some parts of the western world. However, they remain a favored food resource in other parts such as the African and Asian continents. Recently, the increasing demand for GALS of *Archachatina marginata* as a food sup-

ply have intensified effort to enlarge local snail farming scales. However, the lack of sufficient knowledge on critical physiology necessary to boost their reproduction has been interfering with cultivating GALS successfully. Facts relating to health indicators are not well understood and are not readily available to farmers. Provision of relevant information in this area is seriously needed both at local and international levels to further improve farmer's earning and livelihood.

Understanding of GALS's haemolymph chemistry is a fundamental measure for the ultimate establishment of snail farming protocol. The aim of this study was to determine the effect of liveweight on the haemolymph biochemical and physiological parameters in three liveweight categories of *A. marginata* using non-lethal method of hemolymh collection (AJIBOYE 2021) and to develop a set of reference standards for the three weight categories considered.

Materials and Methods

Experimental design and management of animals

Sixty snails used for this study were sourced locally in Abeokuta, Ogun State, Nigeria. The snails were assigned to 3 groups of 20 snails each based on their liveweights (Treatment 1: 100–200 g, Treatment 2: 201–300 g, Treatment 3: 301–400 g) which may not be age related. The snails were acclimatized to laboratory environment before the commencement of haemolymph collection.

Haemolymph collection. Four milliliter (4 ml) of haemolymph was collected from the foot sinus using a 23 Gage needle and syringe. Haemolymph collected was thereafter analyzed to obotain biochemical profiles of hormons and selected minerals.

Haemolymph collection was carried out after cleaning the snails with sterile water. Detailed demonstration of haemolymph collection is shown in Figure 1 (AJIBOYE et al. 2022).

Biochemical analysis of Haemolymph. Total protein and albumin concentrations of each individual were determined using the biuret method described by HENRY et al. (1974). The glucose content was determined by the colorimetric method of BAUMNIGER (1974). The Triglycerides (Trig) and Cholesterol (Chl) assay were done following the method of GRANT (1987). Alanine transaminase (ALT), Aspartate transaminase (AST), and Creatinine were also determined by the methods described by BERGMEYER et al. (1985, 1986).

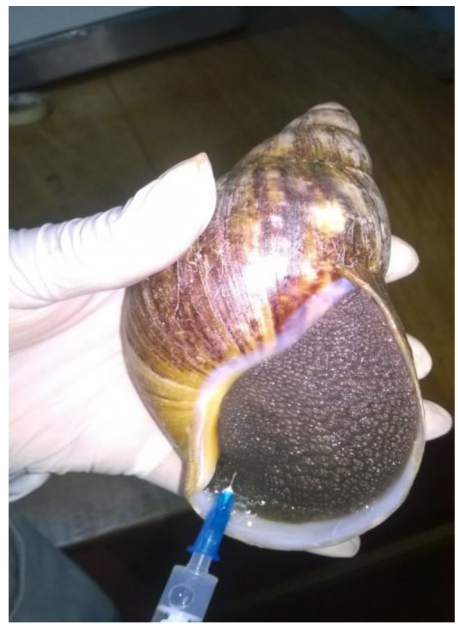

Fig. 1. Demonstration of haemolymph collection via the foot sinus with needle and syringe
Source: AJIBOYE et al. (2022)

Hormonal profile determination. Haemolymph concentrations of total trioiodothyronine (T_3) and tetraiodothyronine (T_4) as well as estradiol (E_2), progesterone (P_4) and follicle stimulating hormone (FSH) were determined quantitatively using commercial Bio-inteco ELISA kits after which results were read with ELX800 Elisa reader.

Selected haemolymph mineral determination. Selected haemolymph minerals like Zinc (Zn), Copper (Cu) and Iron (Fe) were determined spectrophotometrically by appropriate LABKIT.

Results

Effect of liveweight on haemolymph biochemical parameters of *A. marginata* is shown in Table 1. Liveweight had significant effect ($P < 0.05$) on the amounts of albumin, globulin, creatinine, cholesterol and aspartate aminotransferase. On the contrary, liveweight had no significant effect ($P > 0.05$) on those of total protein, glucose, alanine aminotransferase and triglyceride. Albumin concentration was found to be higher in both 100–200 g and 201–300 g liveweight group categories than in the 301–400 g group. The concentration of globulin was significantly higher in

the 300–400 g group than in the 100–200 g and 201–300 g groups. The pattern seen in creatinine was similar to those observed in albumin, but with the exception that the group of snails under 100–200 g were not significantly different from the 301–400 g. For cholesterol, the 301–400 g group had higher significant value than the 100–200 g and 201–300 g groups. AST values were significantly higher in snails of the 100–200 g and 201–300 g groups than those of the 301–400 g group.

Table 1

Effect of liveweight group on haemolymph biochemical parameters of *Archachatina marginata*

LWG [g]	Haemolymph Biochemical Parameters (±SE)								
	TP [g/dl]	Alb [g/dl]	Glb [g/dl]	Glc [g/dl]	Crt [mg/dl]	Chl [mg/dl]	AST [U/L]	ALT [U/L]	Trig [mg/dl]
100–200	3.61 ±0.19	1.86 ±0.1^{a}	1.75 ±0.12^{b}	14.36 ±0.93	1.57 ±0.20^{ab}	22.11 ±1.16^{ab}	64.20 ±3.6^{a}	20.67 ±1.83	18.02 ±2.82
201–300	3.73 ±0.19	2.13 ±0.1^{a}	1.62 ±0.12^{b}	14.69 ±0.93	1.90 ±0.20^{a}	19.65 ±1.16^{b}	63.87 ±3.6^{a}	19.80 ±1.83	23.75 ±2.82
301–400	3.46 ±0.19	1.17 ±0.1^{b}	2.28 ±0.12^{a}	16.57 ±0.93	1.17 ±0.20^{b}	25.25 ±1.16^{a}	46.27 ±3.6^{b}	22.47 ±1.83	17.59 ±2.82

Explanations: ^{ab} means with different superscripts within the same column differ significantly ($p < 0.05$); TP – total protein; Alb – albumin; Glb – globulin; Glc – glucose; Crt – creatinine; Chl – cholesterol; ALT – alanine aminotransferase; AST – aspartate aminotransferase; Trig – triglyceride; LWG – liveweight group

Effect of liveweight on haemolymph hormonal profile of *A. marginata* is shown in Table 2. It was clear from this table that livewight had no significant effect ($P > 0.05$) on follicle stimulating hormone (FSH), trioiodothyronine (T_3), estradiol (E_2) and progesterone (T_4). However, the value for T_4 was higher in 301–400 g than 100–200 g and 201–300 g ($P < 0.05$).

Table 2

Effect of liveweight group on haemolymph hormonal profile of *Archachatina marginata*

LWG [g]	Haemolymph hormonal profile (±SE)				
	FSH [mlU/ml]	T_4 [µg/dl]	T_3 [µg/dl]	E_2 [pg/ml]	P_4 [ng/ml]
100–200	29.53 ±0.50	1.32 ±0.17^{b}	1.51 ±0.12	6.64 ±0.20	2.05 ±0.14
201–300	30.31 ±0.50	1.30 ±0.17^{b}	1.29 ±0.12	7.08 ±0.20	1.79 ±0.14
301–400	30.76 ±0.50	2.90 ±0.17^{a}	1.13 ±0.12	7.17 ±0.20	1.72 ±0.14

Explanations: ^{ab} means with different superscripts within the same column differ significantly ($P < 0.05$); FSH – follicle stimulating hormone; T_4 – tetraiodothyronine; T_3 – trioiodothyronine; E_2 – estradiol; P_4 – progesterone; LWG – liveweight group

Table 3 shows the effect of liveweight on the quantities of selected minerals in the haemolymph of *Archachatna marginata*. Liveweight had significant effect ($P < 0.05$) on copper (Cu) and iron (Fe). However, for zinc (Zn), effect of liveweight was not significant ($P > 0.05$).

Table 3

Effect of liveweight group on selected minerals in *Archachatina marginata*

LWG [g]	Minerals (±SE)		
	zinc (Zn) [µg/dl]	copper (Cu) [µg/dl]	iron (Fe) [µg/dl]
100–200	397.40 ±33.90	505.90 ±17.90^{a}	372.90 ±30.80^{a}
201–300	414.70 ±33.90	471.30 ±17.90^{a}	295.70 ±30.80^{ab}
301–400	320.40 ±33.90	302.10 ±17.90^{b}	204.40 ±30.80^{b}
Reference	300–450	250–480	204–380

Explanations: ^{ab} means with different superscripts within the same column differ significantly ($P < 0.05$); LWG – liveweight group

Discussion

Liveweight had no significant effect on haemolymph total protein. The results on protein concentration in the three size groups of *Indoplanorbis exustus* and *Lymnaea acuminate F. rufescens* showed an increasing trend in relation to size/age. Other factors such as parasitism, bacterial challenge and starvation are considered very important factors controlling total haemolymph protein in molluscs (REJU 1990). The level of serum protein in *B. glabrata* was found to increase when challenged with live bacterium (CHENG et al. 1978). Haemolymph protein and free amino acid concentrations in *B. glabrata* were found to change due to infection (DUSANIC and LEWERT 1963, GILBERTSON et al. 1967, LEE and CHENG (1972). By day 70 post-exposure to the parasite *Schistosoma mansoni*, the total protein content had declined to one third of that in uninifected snails (GRESS and CHENG 1973, MANOHAR and RAO 1977). BECKER and HIRTBACH (1975) reported decrease in haemolymph protein after seven days of starvation in *B. glabarata*.

In snails, it has been identified that the lipids are involved in the animal's survival under physiological stress conditions, such as long feed restriction or when snails are parasitized, when the carbohydrates reserve are quickly depleted and the lipids are consumed more frequently (STOREY 2002, GIOKAS et al. 2005, BANDSTRA et al. 2006). LUSTRINO et al. (2010) reported that triglyceride metabolism in *A. fulica* is more influenced by photoperiod variations. REJU (1990) reported significant variation in total lipid concentration among snails of different size groups in *P. virens*. Lipid level was found to be significantly higher in small size groups than in the intermediate and larger groups. Considering the intermediate and larger groups as adults and hence reproductively very active, he attributed the low values to the utilization of lipid during gametogenesis. GOMOT (1998)

also reported a decrease in the lipid content of *H. pomatia, H. aspersa* and *H. aspersa maxima* with age. Lipids are the metabolic storage products for producing gametes (WEBBER 1970) hence gametogenesis leads to a decline in lipid storage content which is reflected as low haemolymph lipid level in adults. The high haemolymph lipid content in small size group snails may be an indication of large scale movement of lipid to gonads prior to gametogenesis (REJU 1990). NICOLAI et al (2012) reported difference in triglyceride concentrations between physiological states but not between geographical origins in the land snail *Helix aspersa*. Contrary to Reju's findings, the results of this study indicated that liveweight had no significant effect on the concentration of triglycerides in *A. marginata* snails. Differences in synthesis and utilization of lipids between species may be responsible for the results obtained. The physiological state of the snails used may also be responsible for the difference in results obtained.

Alanine aminotransferase concentration was not significantly affected by liveweight. Aspartate aminotransferase was, however, significantly affected. This result corroborates the statement by SWAMI and REDDY (1978), that the size of the animal is one of the factors affecting transaminase activity. There was no significant difference between AST concentrations between liveweight group 1 and 2 (100–200 g and 201–300 g) snails with the concentration being higher in the liveweight group 3 (301–400 g) snails. REJU (1990) reported a decline in AST and ALT activity with increase in body size. According to him, the decrease was more prominent in ALT. The author attributed the decline in the activity of these enzymes to the general decrease of metabolic rate and growth rate noted in gastropods along with increase in size or progress in age (HANIFFA 1980; ALDRIDGE 1982). In small size group snails, increased metabolic and assimilation rates require transaminase in high concentrations while with decrease in metabolic turnover, in large snails, the levels of transferases also show a declining trend (REJU 1990). Other factors affecting the transaminase activity such as difference in species, season, food, size of the animal, and assay procedures (SWAMI and REDDY 1978) may be responsible for the differences in results obtained for ALT in these snails.

Liveweight had no significant effect on haemolymph glucose concentration. This result is contrary to that of SURESH (1990). Suresh reported considerable variations in total carbohydrate and glycogen levels in the three size groups of both *I. exustus* and *L. acuminate, F. rufescens*. In both species, a general trend for increase in the concentrations of both total carbohydrate and glycogen was observed in relation to the size/age of the animals. In both species, the largest size group showed highly significant increase in glycogen and carbohydrate levels than the other two size

groups. According to him, it may be considered that age/shell size is a factor which to a certain extent determines the level of both glycogen and total carbohydrate in *I. exustus* and *L. acuminata.* Haemolymph glucose concentration in *A. marginata* does not seem to be weight dependent as can be deduced from the results of this study. Numerous factors have been reported to alter the haemolymph glucose concentrations in gastropods. They include (a) blood sampling procedures and other experimental manipulations (SUBRAMANYAM 1973), (b) quality of food assimilated (MEENAKSHI and SCHEER 1968, FRIEDL 1971, SCHEERBOOM 1978, STANISLAWSKI and BECKER 1979), and (c) quantity of food (SCHEEERBOOM 1978), (d) seasonal changes; the changes are usually in response to changes in environmental temperature and food availability and are often linked to seasonal reproductive cycles, (e) aestivation, which is characterized by a drop in oxygen consumption and haemolymph reserve carbohydrate (SWAMI and REDDY 1978, HORNE 1979), (f) circadian flunctuations, for instance in the slug *L. alte*, the total carbohydrate of several tissues was the highest during the inactive light phase and lowest during the active dark phase (KUMAR et al. 1981) and [g] parasitism; decreased tissue carbohydrate levels and/or decreased blood glucose concentrations as a result of infection in molluscs have been observed in a number of cases (LEE and CHENG 1971, ROBSON and WILLIAMS 1971, CHRISTIE et al. 1974, VAIDYA 1979, MOHAMED and ISHAK 1981).

The presence of thyroid hormones was reported for the first time in *A. fulica* a land gastropod by LUSTRINO et al (2017). Thyroid hormones and their receptors have been well studied in vertebrates but less is known about the mechanisms by which they regulate the physiology and behavior in molluscs (LUSTRINO et al. 2017). The presence of TSH receptors and the evidence of endogenous synthesis of TSH have been shown in the freshwater gastropod *Lymnaea stagnalis* (TENSEN et al. 1994), the sea hare *Aplysia californica* (HEYLAND et al. 2006), Pacific Oyster, *Crassostrea gigas* (Huang et al. 2015). According to LUSTRINO et al (2017) only T_4 was found in *A. fulica* by Radioimmunoassay (RIA) analysis and its level declined after both starvation and deproteinisation. According to them, T_4 concentrations varied greatly among the animals used for the experiment. Also, no significant correlation was found between the biomass of the molluscs and T_4 concentration. In this study, liveweight had no significant effect on T_3. It, however, had significant effect on T_4 in the larger snails (301–400 g). There was no significant difference in the liveweight effect on the smaller snails (100–200 g, 201–300 g).

Liveweight had no significant effect on FSH, P_4 and E_2. According to OKHALE et al (2018), the mean concentrations of the hormones were found to be significantly influenced by the reproductive phases and aestivation of

the snails. DI COSMO et al. (2001), reported that progesterone levels flunctuate according to the reproductive cycle in molluscs being very low during the non-vitellogenic period and increasing at the onset of vitellogenesis. OKHALE et al (2018) reported that FSH was relatively unaffected by aestivation treatments across reproductive phases. The author, however, reported that there was a trend of higher concentration of FSH at the dormancy and a decline in the pre-spawning and spawning phases and an eventual upsurge at the post-spawning phase. Environmental conditions affect reproductive function in live snails (CELIK et al. 2019). Optimal temperature, humidity and photoperiod encourage breeding activity of *C. aspersum* (JEPPESEN and NYGARD 1976, DAGUZAN 1982). From the current result, it may be inferred that these snails used in their study were probably not in the reproductive phase.

Liveweight had significant effect on Cu and Fe concentrations but had no significant effect on Zn concentration. This finding is in agreement with LUKONG et al. (2012). LUKONG et al. 2012, investigated the effect of prolonged aestivation on the composition of the major elements (K, Na, Ca, Mg, Fe and Zn) and found that the concentration of Zn did not change significantly throughout the experimental procedure while concentrations of Fe showed significant changes.

Accepted for print 26.11.2024

References

ABDUSAMMAD A.M. 2010. *Effect of duration of aestivation on haemolymph biochemical and biophysical parameters of two liveweight groups of Archachatina marginata Swainson*. 1821. Veterinaria, 4(4): 400–408.

ABIONA J.A., ABIOJA O.M., AYO-AJASA O.Y., OYESANWEN M.T., IHONGBE R.A., MSHELBWALA F.M., OKE O.E., ONAGBESAN O.M. 2021. *Effect of common salt (NaCl) on reproductive tract, biochemical parameters of Giant African Land Snail (A. marginata) during dry season*. Aquaculture Reports, 20.100695.

ABIONA J.A., AKINDUTI P.A., OSINOWO O.A., ONAGBESAN O.M. 2013. *Comparative evaluation of inhibitory activity of epiphragm from albino and normal skinned Giant African Land Snail (A.marginata) against selected bacteria isolates*. Ethiopian Journal of Environmental Studies and Management, 6(2): 177–181.

ABIONA J.A., AKINDUTI P.A., OYEKUNLE M.A., OSINOWO O.A., ONAGBESAN O.M. 2014. *Comparative evaluation of haemagglutination potential of haemocytes from two species of Giant African Land Snails* (*Archachatina marginata and Achatina achatina*). Fish shellfish Immunology, 38(1): 96–100

ABIONA J.A., OJO O., LADOKUN A.O., ABIOJA M.O., DARAMOLA J.O., JAMES I.J., OSINOWO O.A., ONAGBESAN O.M. 2016. *Effect of Mucuna pruriens on the reproductive tract of Giant African Land Snail* (*Archachatina marginata*). Nigerian Journal of Animal Science, 18(2): 315–321.

ABIONA J.A., OLAOYE G.T., ABIOJA M.O., ONAGBESAN O.M. 2018. *Effect of Zinc oxide on liveweight, reproductive organ dimensions, spermatozoa production of A. marginata during dry season*. Agricultura Tropica et Subtropica, 51(4): 147–154.

ABIONA J.A., SOKOYA Y.F., OSINOWO O.A., LADOKUN A.O., ABIOJA M.O., ONAGBESAN O.M. 2012. *Effect of egg weight on hatching weight and incubation period in Giant African Land Snail* (*Archachatina marginata*). Nigerian Journal of Animal Science, 14: 181–187.

ADEBAYO M.O. 2011. *Comparative study of the nervous system of Giant African Land Snails* (*Archachatina marginata and Achatina achatina*). M. Agric. Thesis, Department of Animal Physiology, Federal University of Agriculture, Abeokuta.

ADIO B.R. 2010. *Effects of induced humidity on liveweight changes, sizes of reproductive organs and egg laying of Archachatina marginata* during the dry season. M. Agric. Thesis, Department of Animal Physiology, Federal University of Agriculture, Abeokuta.

AGRAWAL A., MITRA S., GHOSH N., BHATTACHARYA S.1990. *C-reactive protein [CRP) in haemolymph of a mollusc, Achatina fulica Bowdich*. Indian Journal of Experimental Biology, 28: 78–89.

AJIBOYE O.O., ABIONA J.A., ONAGBESAN O.M., DUROTOYE V.L. 2022. *Effect of site of collection and liveweight on hemolymph volume and survival of Archachatina marginata*. Journal of Molluscan Research, 28: 97–106.

ALDRIDGE S.A. 1982. *Drug induced sexual dysfunction*. Clinical Pharmacology, 2: 141–147.

ALLAM B., PAILLARD C., HOWARD A., LE PENNEC M. 2000. *Isolation of the pathogen Vibrio tapetis and defense parameters in brown ring diseases Manila clams Ruditapes philippinarum cultivated in England*. Diseases of Aquatic Organisms, 41: 105–113.

BANDSTRA S.R., FRIED B., SHERMA J. 2006. *High-performance thin-chromatographic analysis of neutral lipids and phospholipids in Biomphalaria glabrata patently infected with Echinostoma caproni*. Parasitology Research, 99(4): 414–418.

BAUMNIGER R.N. 1974. *Analytical Chemistry. Cambridge Press, London*, pp. 83–85.

BERGMEYER H.U., HORDER M., REJ R. 1986. *Approved recommendation (1985) on IFCC methods for the measurement of catalytic concentration of enzymes. Part 3. IFCC Method for aspartate aminotransferase*. J. Clin. Chem. Clin. Biochem., 24: 481–489.

BERGMEYER H.U., HORDER M., REJ R .1985. *Approved recommendation on IFCC methods for the measurement of catalytic concentration of enzymes. Part 3, IFCC Method for alanine aminotransferase*. J. Clin. Chem. Clin. Biochem., 33: 321–238.

BROCKELMAN C.R. 1975. *Inhibition of Rhabditis maupasi* (Rhabditidae:nematoda), *maturation and reproduction by factors from the snail host*, *Helix aspersa*. Journal of Invertebrate Pathology, 25: 229–37.

BROCKELMAN C.R., SITHITHAVORN P. 1980. *Carbohydrate reserves and haemolymph sugars of the African giant snail Achatina fulica in relation to parasitic infection and starvation*. Zeitschrift fUI Parasitenkunde, 62: 285–91.

BROCKELMAN C.R. 1978. *Effects of parasitism and stresson haemolymph protein of the African giant snail ‚Achatina fulica*. Zeitschrift fiir Parasitenkunde, 57: 137–44

CELIK M.Y., DUMAN M.B., SURHPEK M., UZUN GOREN G., KAYAOTURK D., KARAYUCEL S. 2019. *Growth and Mortality rates of Cornu aspersum*: *organic snail culture, Black sea region*. Journal of Agricultural Science, 25:189–196.

CHENG T.C., GUIDA V.G., GERHART P.L. 1978. *Aminopeptidase and lysozyme activity levels and serum protein concentrations in Biomphalaria glabrata* (*Mollusca*) *challenged with bacteria*. Journal of Invertebrate Pathology, 32: 297–302.

CHRISTIE J.D., FOSTER W.B., STAUBER L.A. 1974. *The effect of parasitism and starvation on carbohydrate reserves of Biomphalaria glabrata*. Journal of Invertebrate Pathology, 23: 297–302.

COOK A. 1989. *Slugs and snails*. Biologist, 36: 183–8.

COOPER J.E., KNOWLER C. 1991. *Snails and snail farming: an introduction for the veterinary surgeon*. Veterinary Record, 129: 541–549.

DAGUZAN J. 1982. *Contribution á l'élevage de l'escargot Petit-gris Helix aspersa* Müller. 2. Ann. Zootech., 31(2): 87–110.

DI COSMO A., DI CRISTO C., PAOLUCCI M. 2001. *Sex steroid hormone fluctuations and morphological changes of the reproductive system of the female of Octopus vulgaris throughout the annual cycle*. Journal of Experimental Zoology, 289: 33–47

DORAN W.J., COPE W.G., RADA R.G., SANDHEINRICH M.B. 2001. *Acetylcholinesterase inhibition in the ridge mussel* (*Amblema plicata*) *by* chlorpyrifos: *implications for biomonitoring*. Ecotoxicology and Environmental Safety, 49: 91–98.

DUSANIC, G.D. AND LEWERT, R.M. 1963. *Alterations of proteins and free aminoacids of Australorbis glabratus haemolymph after exposure to Schistosoma mansoni miracidia*. Journal of Infectious Diseases, 112: 243–246.

FELDMAN B.F., JINKL J.G., JAIN J.C. 2000. *Schalm's veterinary hematology, 5th edn*. Lippincott Williams and Wilkins, Philadelphia, PA.

FRIEDL F.E. 1971. *Haemolymph glucose in the freshwater pulmonate snail Lymnaea stagnalis Basal values and an effect of ingested carbohydrate*. Comparative Biochemistry and Physiology, A 39A: 605–610.

GILBERTSON D.E., ETGES F.J., OGLE J.D. 1967. *Free aminoacids of Australorbis haemolymph: Comparison of four geographic strains and effect of infection by Schistosoma mansoni*. Journal of Parasitology, 53: 565–568.

GIOKAS S., PAFILIS P., VALAKOS E. 2005. *Ecological and physiological adaptations of the land snail Albinaria caerulea* (Pulmonata: Clausiliidae). Journal of Molluscan Studies, 71(1): 15–23.

GOMOT A. 1998. *Biochemical composition of Helix snails: influence of genetic and physiological factors*. Journal of Molluscan Studies, 64: 173–181.

GRANT G.H. 1987. *Amino acids and protein: Fundamentals of clinical chemistry*. WB Sander Company, Philadelphia, U.S.A, pp. 326–329.

GRESS F.M., CHENG T.C. 1973. *Alterations in total serum proteins and protein fractions in Biomphalaria glabrata parasitized by Schistosoma mansoni*. Journal of Invertebrate Pathology, 22: 382–390.

GUSTAFON L.I., STOSKOPF M.K., BOGAN A.E., SHOWERS W., KWAK T.J., HANLON S. LEVINE J.F. 2005a. *Evaluation of a non-lethal technique for haemolymph collection in Elliptio complanata, a freshwater bivalve* (Mollusca: Unionidae) Diseases of Aquatic Organisms, 65: 159–165.

HANIFFA M.A. 1980. *Studies on life span, growth increments and energy budget of freshwater snail Pila globosa* (*Swainson*) *in an artificial pond*. Proceedings: Animal Sciences, 89(3): 275–286.

HENRY R.J., CANON D.C., WINKALMAN J.W. 1974. *Clinical Chemistry; Principle and Technique 2nd ed*, Harper and Row Pubishers, New York, pp 54–56.

HORNE F.B. 1979. *Comparative aspects of an aestivation metabolism in the gastropod*, *Marisa cornuarietis*. Comparative Biochemistry and Physiology, A 64A: 309–312.

JEPPESEN L.L., NYGARD K. 1976. *The influence of photoperiod, temperature and internal factors on the hibernation of Helix pomatia* L. (Gastropoda, Pulmonata). Vidensk Meddr Dansk Natuhr Foren, 139: 7–20.

KUMAR T.P., RAMAMURTHI R., BABU K.S. 1981. *Circadiae fluctuations in total protein and carbohydrate content in the slug Laevicaulis alte* (*Ferrussac*,1821). Biological Bulletin (Woods Hole, Mass). 160: 114–122.

LEE F.O., CHENG T.C. 1972. *Schistosoma mansoni infection in Biomphalaria glabrata*: *alterations total protein and hemoglobin concentration in the host's haemolymph*. Experimental Parasitology, 31: 203–216.

LUKONG C.B., EZEBUO F.C., VERLA A.W. 2012. *Characterization of the major essential elements in the Haemolymph of the Giant African Land Snail (Achatina achatina) During Aestivation*. Biosciences Biotechnology Research Asia, 9(2): 577–584.

LUSTRINO D., SILVA C.M., ARAUJO G.I., TUNHOLI V.M., TUNHOLI-ALVES V.M., CASTRO R.N., CARVALHO D.P., PINHEIRO J., MARASSI M.P. 2017. *Evidence of the presence of thyroid hormones in Achatina fulica Snails*. Annals of the Brazilian. Academy of Sciences 89(3): 2181–2188.

LUSTRINO D., TUNHOLI-AVES V.M., TUNHOLI V.M., MARASSI M.P., PINHEIRO J. 2010. *Lipids analysis in haemolymph of African Giant Achatina fulica* (*Bowdich*, 1822) *exposed to different photoperiods*. Brazilian Journal of Biology, 70(1): 129–134.

MALEV O., SRUT M., MAGUIRE I., STAMBUK A., FERRERO E.A, LORENZON S., KLOBUCAR G.I. 2010. *Genotoxic physiological and immunological effects caused by temperature increase, air exposure or food deprivation in fresh water crayfish Astacus leptodachylus*. Comparative Biochemistry and Physiology, Part C. Toxicology and Pharmacology, 152: 433–443.

MANOHAR L., RAO P.V. 1977. *Physiological response to parasitism, Part II. Gluconeogenic precursor levels and related enzyme activity profiles in the snail host*. Indian Journal of Experimental Biology, 15: 264–267.

MEENAKSHI V.R., SCHEER B.T. 1968. *Studies on the carbohydrates of the slug Ariolimax columbianis with special reference to their distribution in the reproductive system*. Comparative Biochemistry and Physiology, 26: 1091–1097.

MEKA J.M., MCCORMICK S.D. 2005. *Physiological responses of wild rainbow trout to angling: impact of angling duration, fish size, body condition and temperature*. Fisheries Research, 72: 311–322.

MOHAMED A.M., ISHAK M.M. 1981. *Growth rate and changes in tissue carbohydrates during schistosome infection of the snail Biomphalaria alexandrina*, Hydrobiologia, 76: 17–21.

NICOLAI A., FILSER J., LENZ R., BERTRAND C., CHARRIER M. 2012. *Quantitative Assessment of Haemolymph Metabolites in two physiological states and two populations of the land snail H. pomatia*. Physiological and Biochemical Zoology, 85(3): 274–284.

OKHALE O.E., OMOYAKHI O., TAIWO E., AREMU O.T. 2018. *Hormonal Concentration of Controlled and Naturally Induced Aestivated Snails (Archachatina marginata) at Different Reproductive Phases*. *Journal of Applied Life Sciences International*, 16(1): 1–9.

PAILLARD C., ASHTON-ALCOX K.A., FORD S.E. 1996. *Changes in bacterial densities and hemocyte parameters in eastern oysters, Crassostrea virginica, affected by juvenile oyster disease*. Aquatic Living Resources, 9:145–158.

REJU M.K. 1990. *Studies on haemolymph constituents of pila virens (Lamarck) and the effects of pesticides on the activity pattern of selected enzymes. PhD thesis*, Faculty of Environmental Studies, Cochin University of Science and Technology.

RENWRANTZ L., SPIELVOGEL F. 2011. *Heart rate and haemocyte number as stress indicators in disturbed hibernating vineyard snails, Helix pomatia*. Comparative Biochemistry and Physiology. Part A: Molecular and Integrative Physiology, 160: 467–473.

ROBSON E.M., WILLIAMS I.C. 1971. *Relationships of some species of digenea with the marine prosobranch Littorina littorea* (L.) Ill. *The effect of larval digenea on the glycogen content of the digestive gland and foot of L. littorea*. Journal of Helminthology, 45: 381–401.

SALAMI M.I. 2010. *Study of the Circulatory System and effect of aestivation on heart rate of Archachatina marginata, Swainson*1821 *at different liveweights*. M. Agric. Thesis, department of Animal Physiology, Federal University of Agriculture, Abeokuta.

SCHEERBOOM J.E.M. 1978. *The influence of food quantity and food quality on assimilation, body growth and egg production in the pond snail Lymnaea stagnalis* (*L.*) *with particular reference to the hernolymph – glucose concentration*. Koninklijke Nederlands Akademic van Wetenschappen. Series C 81: 184–197

SODIPE O.G. 2011. *Comparative study of the haemolymph of the Giant African Land Snail, Archachatina marginata*. PhD Thesis, Department of Animal Physiology, Federal University of Agriculture, Abeokuta.

STANISLAWSKI E., BECKER W., MULLER G. 1979. *Alterations of the free amino acid content in the haemolymph of Biomphalaria glabrata* (Pulmonata) *in starvation and after infection with Schistosoma mansoni* (Trematoda). Comparative Biochemistry and Physiology, B 63B: 477–482.

STOCKHAM S.L., SCOTT M.A. 2002. *Fundamentals of Veterinary Clinical Pathology*. Iowa State Press, Ames, IA.

STOREY K.B. 2002. *Life in the low lane: molecular mechanisms of estivation*. Comparative biochemistry and Physiology, A 133(3): 733–754.

SUBRAMANYAM O.V. 1973. *Neuroendocrine control of metabolism of a pulmonate snail*. Ariophanta sp. Experientia, 29: 1150–1151.

SURESH P.G., MOHANDAS A. 1990. *Studies on haemolymph constituents of indoplanorbis exustus (Deshayes) and Lymnaea acuminata* (Larmarck) rufescens (*gray) and the effects of copper on the activity pattern of selected transaminases and phosphatases*. PhD thesis. School of Environmental Studies, Cochin University of Technology, Cochin.

SWAMI K.S., REDDY Y.S. 1978. *Adaptive changes to survival during aestivation in the gastropod snail, Pila globosa*. Journal of Science and Industrial Research, 37: 144–157.

TENSEN C.P., VAN KASTEREN E.R., PLANTA R.J., COX K.J., BURKE J.F., VAN HEERIKHUIZEN H., VREUGDENHIL E. 1994. *A G protein coupled receptor with low-density lipoprotein-binding motifs suggests a role for lipoproteins in G-linked signal transduction*. Proceedings of the National Academy of Sciences, 91: 4816–4820.

AJIBOYE O.O., ABIONA J.A., ONAGBESAN O.M., DUROTOYE V.L. 2022. *Effect of site of collection and liveweight on hemolymph volume and survival of Archachatina marginata*. Journal of Molluscan Research, 28: 97–106.

VAIDYA D.P. 1979. *The pathological effects of trematodes on the biochemical components of hepatopancreas and blood glucose of freshwater snail Indoplanorbis exustus Riv*. Parasitology, 40: 267–272.

WEBBER H.H. 1970. *Changes in metabolic composition during the reproductive cycle of the abalone Haliotis cracheroidii* (*Gastropod: Prosobranchiata*). Physiological Zoology, 43(3): 213–231.

WOBESER G.A.1994. *Investigation and management of disease in wild animals*. Plenum Press, New York.