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Short communication:

Growth biometrics and DNA yield of *Bulinus* snail from River Wudil, Nigeria

Rasheed Olatunji Moruf [1*], Halima Abdullahi Muhammad [2]

[1]*Department of Fisheries and Aquaculture, Bayero University, P.M.B. 3011, Kano, Nigeria.*
[2] *Department of Animal Science, Bayero University, P.M.B. 3011, Kano, Nigeria.*

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ABSTRACT

The measurement and analysis of snail biometrics help to identify and differentiate species, study their growth, and understand their ecological relationships. The growth pattern, DNA yield and purity of Bulinus. globosus from River Wudil, Kano State, were investigated using standard methods. The mean ranges in mm and g from 7.9±2.9, 5.7±2.5, 4.8±1.8, 3.4±1.2, 1.5±0.2, 1.6±0.0, 2.4±0.2, 1.2±0.1, 1.4±0.1, 65.8±1.9 and 22.2±0.4 were recorded for length, width, aperture height, aperture width, height width ratio, height and aperture height ratio, height and aperture width ratio, width and aperture height ratio, aperture height and aperture width ratio, shell weight and flesh weight respectively. About 33.7 % of weight of the snail is made up of flesh by weight. The regression coefficient "b" showed that B. globosus exhibited a negative allometric growth pattern while the correlation coefficient (r) in all the shell parameters were below 1, indicating a weak correlation between the parameters. Mean DNA yield and purity were 120.03 ± 5.10 ng/µl and 1.81 ± 1.21. It is concluded that the freshwater snail, B. globusus from River Wudil exhibits negative allometric growth pattern and its DNA yield is well above the minimum standard (A260/A280 ratio of 1.7–2.0) while the pure extracts are good enough for further molecular study.

Introduction

Freshwater snails are a prominent component of numerous important ecological community groups. They are found to be both commercially and medicinally advantageous (Supian and Ikhwanuddin, 2017). They benefit man by providing him with food, jewelry, tools, and even pets. Freshwater snails are important for both human and animal health. In the food chain, freshwater snails play a key role as primary consumers because they transform microbes, plants, fungi, and decaying matter into a source of food that may be used by a wide range of animals, including fish, amphibians, reptiles, birds and mammals (Auta *et al.*, 2018). Waterfowl, amphibians, turtles, and fish like sculpins and trout all eat freshwater snail.

Bulinus is a genus of small tropical freshwater snails, aquatic gastropod molluscs in the family Bulinidae and are widely distributed throughout much of Sub-Sahara Africa (WHO, 1995). Species in this genus inhabit various natural and artificial freshwater environments including shallow lakes, streams, rivers, wetlands, seasonal pools, rice paddies, irrigation canals and ponds (Falade and Otarigho, 2015). Meanwhile, species within the genus *Bulinus* have been placed into four groups: *Bulinus africanus, Bulinus forskalii, Bulinus reticulatus* and *the Bulinus truncatus/tropicus* complex (Kane *et al.*, 2008). For the most part, species have been classified on the basis of their morphology although, in recent decades, the study of ploidy, allozymes and DNA methods have all played an increasing role in species discrimination (Moruf and Adekoya, 2020). Morphological characters, whilst adequate to allocate a specimen to a species group are sometimes unreliable when used to classify at higher resolution especially within the *Bulinus africanus* group (Zhang *et al.*, 2022).

* Corresponding author.
Email address: tunjimoruf@gmail.com

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Several works of literature are available on *Bulinus* species in terms of ecology, feeding, shell morphology and molecular characterization among others (Akinwale *et al*., 2011; Agbolade *et al*., 2013; Akinwale *et al*., 2015; Falade and Otarigho, 2015; Hang *et al*., 2022). Shell morphometric is useful tool and first step in identification in mollusc taxonomy and ecological studies. In shellfish biology, shell morphology has been useful in describing, identifying, characterizing and recognizing intraspecific morphological variations (Moruf and Lawal-Are, 2017; Moruf, 2020). It has also assisted in deducing shell structures and properties affected by environmental variations to determine snail geographical distribution (Falade and Otarigho, 2015).

With the establishment of techniques that specifically verify biochemical or molecular genetic variation, the orthodox methods still have an important role in identifying stock till the present times (Asaduzzaman *et al*., 2021). However, for effective management of populations, there is a need to understand snail taxonomic identity. Hence, the goal of this study was to assess the morphological features of *Bulinus africa*n in River Wudil, Nigeria by using a combination of both biometric and DNA techniques. This will serve as a baseline study for biologists in future research on the *Bulinus* snail.

Materials and Methods

Study location

River Wudil is a tributary of River Hadejia and gets it water from River Challawa, River Kano and their tributaries. It flows northeast as River Hadejia and finally into Lake Chad. The river is an important part of the Hadejia and the Jama' are river system. The River Wudil is situated in Wudil Local Government Area, east-central area of Kano State Nigeria, with an estimated land area of 458 km2 with longitude 8° 45′ E and 8° 57′ E, as well as between latitude 11° 37′ N and 11° 56′ N (Getso *et al*., 2017). The river basin occupies a total land area of about 16386.0136 km^2 and is part of the inland drainage system of the Chad Basin (Olofin, 2005)

Collection of sample

A total of 185 specimens of *B. globosus* were collected from water contact sites in River Wudil. The specimens were collected alive using a standard snail scoop, the contents washed and the snails picked manually. The recovered snails were transported in pre-labelled plastic containers to the laboratory of the Department of Fisheries and Aquaculture, Bayero University, Kano. Each snail was identified based on its morphological characteristics using the field guide to African freshwater snails by Kristensen (1987). The samples for molecular studies were preserved in 70% ethanol and further analysed in conjunction with the African Bioscience Limited in Ibadan, Nigeria..

Laboratory procedures

For the biometrics, the weight was taken to the nearest 0.1 g on a Sartorius top loading balance (Model: DT1001A). while the following linear measurements were recorded using a Vernier caliper: shell height (H), shell width (W), shell aperture height (AH), and shell aperture width (AW) according to Chiu et al. (2002). From the values obtained for each linear measurement, the following ratios were calculated: shell height/shell width (H/W); shell height/aperture height (H/AH); shell height/aperture width (H/AW); shell width/aperture height (H/AW) and aperture height/aperture width (AH/AW).

In terms of molecular studies, DNA were extracted using quick-DNATM Universal kit (2R D4068) manufactured by Zymo Research, following the procedures as specified by the manufacturer, following these six steps Spectrophotometric analysis was done by measuring the concentrations and relative absorbance of the DNA solutions extracted from the snail. It is employed to ascertain the purity and concentration of a given DNA sample. A micropipette was used to measure 95 µl of distilled water into a cuvette for blank checking, followed by the addition of 5 µl of DNA sample and mixed thoroughly without having bubbles. Then cuvette was placed in the Bio-photometer to determine the concentration of the sample, the relative absorbance at a different wavelength (230 nm, 260 nm, 280 nm, and 340 nm respectively) and the absorbance ratio (at wavelength 260 nm to 280 nm) were determined and recorded for each of the samples. One Tag Quick-Load EZ-VISION© Blue Light DNA Dye was used in loading dye and buffer. The procedure for the gel electrophoresis involved checking the quality of DNA samples and it was achieved using 1% Agarose gel, which was prepared by measuring 1.00 g of Agarose (CSL-AG 100) into a beaker and 100ml of 1X TBE buffer was added. It was later placed into the microwave to dissolve the solution for 1-2 minutes and the solution was allowed to cool down before adding 4 µl of Ethidium Bromide and swirl to mix. The quantity of the DNA samples determines the quantity of Agarose gel to prepare.

Data analysis

The mean, standard deviation, minimum, maximum values and the general linear regression were performed on shell character, using statistical package SPSS version 22.0.

Results

Biometry characteristic

The values of tissue weight and shell biometrics of *B. globosus* are presented in Table 1. The ranges in mm and g from 2.1 to 12.0 (7.9±2.9), 1.1 to 9.4 (5.7±2.5), 1.3 to 7.3 (4.8±1.8), 1.0 to 5.1 (3.4±1.2), 1.1 to 2.0 (1.5±0.2) 1.0 to 1.9 (1.6 ± 0.0), 1.1 to 3.6(2.4 ± 0.2), 1.0 to 1.4 (1.2 ± 0.1), 1.1 to 1.7 (1.4 ± 0.1), 26.5 to 103.9 (65.8 ± 1.9) and 10.8 to 35.9 (22.2 ± 0.4) were recorded for height, width, aperture height, aperture width, height width ratio, height and aperture height ratio, height and aperture width ratio, width and aperture height ratio, aperture height and aperture width ratio, shell weight and flesh weight respectively. About 33.7 % of weight of the snail is made up of flesh by weight.

The shell dimension – weight relationships are illustrated in Figures 1 – 5. The exponent "b", varied from 0.4928 (flesh weight – shell length) to 0.6381 (Shell weight – Aperture height).

The value of "b", 0.6381 in Figure 1 showed that *B. globosus* exhibited a negative allometric growth pattern while the correlation coefficient (r), 0.7730 indicates a strong correlation (close to 1) between its shell length and shell weight.

The values of "b" (0.4928) and "r" (0.7981) in Figure 2 showed negative allometric growth and a positive correlation between the shell length and flesh weight.

The value of "b", 0.5717 in Figure 3 represents negative allometric growth whiles the correlation coefficient of 0.8054 indicates a strong correlation (close to 1) between its shell width and shell weight. As shown in Figure 4, the aperture height and shell weight are correlated (b = 0.6381; r = 0.7751).

The value of "b", 0.6341 in Figure 5 represents negative allometric growth while the correlation coefficient of 0.7738 indicates a positive correlation between its aperture width and shell weight.

In this study, the exponents were generally lower than 3, indicating negative allometric growth. The correlation coefficients (r) in all the shell variables were below "1", showing a week correlation between the parameters.

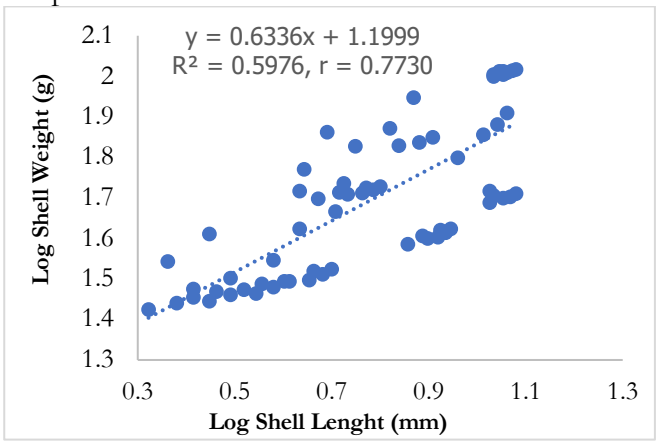

Figure 1. Log shell length – Log shell weight relationship of *Bulinus globusus* from River Wudil, Nigeria.

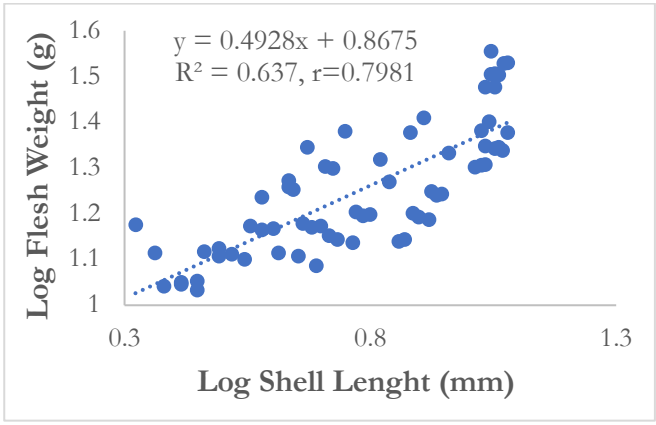

Figure 2. Log shell length – Log flesh weight relationship of *Bulinus globusus* from River Wudill, Nigeria.

Table 1. Growth pattern of the *Bulinus* snail from River Wudil, Nigeria

Dimension	Minimum	Maximum	Mean ± SD
Height (H)	2.1	12.0	7.9 ± 2.9
Width (W)	1.1	9.4	5.7 ± 2.5
Aperture height (AH)	1.3	7.3	4.8 ± 1.8
Aperture width (AW)	1.0	5.1	3.4 ± 1.2
Height Width ratio (H/W)	1.1	2.0	1.5 ± 0.2
Height and Aperture Height ratio (H/AH)	1.0	1.9	1.6 ± 0.0
Height and Aperture Width ratio (H/AW)	1.1	3.6	2.4 ± 0.2
Width and Aperture Height ratio (W/AH)	1.0	1.4	1.2 ± 0.1
Aperture Height & Aperture Width ratio (AH/AW)	1.1	1.7	1.4 ± 0.1
Shell weight (SW)	26.5	103.9	65.8 ± 1.9
Flesh Weight (FW)	10.8	35.9	22.2 ± 0.4

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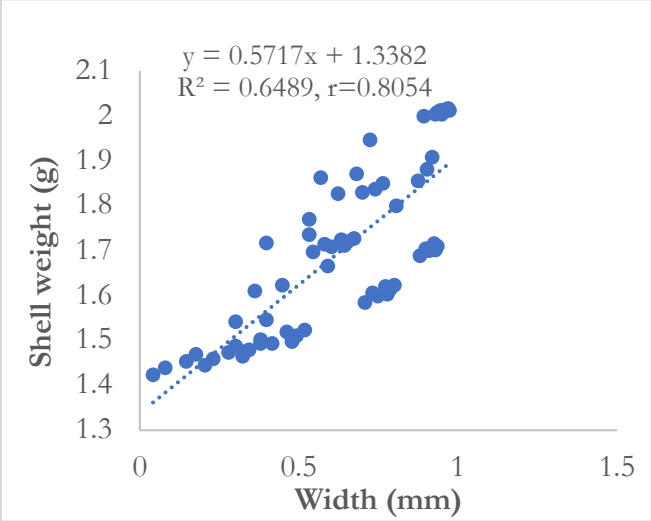

Figure 3. Log shell width – Log shell weight relationship of *Bulinus globusus* from River Wudill, Nigeria.

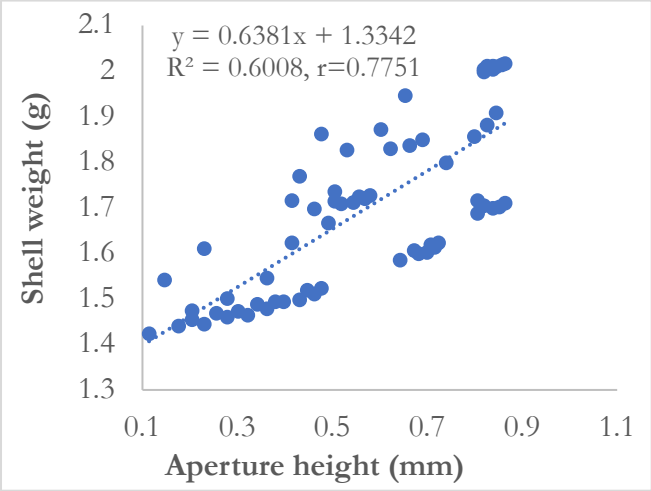

Figure 4. Log Aperture height – Log shell weight relationship of *Bulinus globusus* from River Wudill, Nigeria.

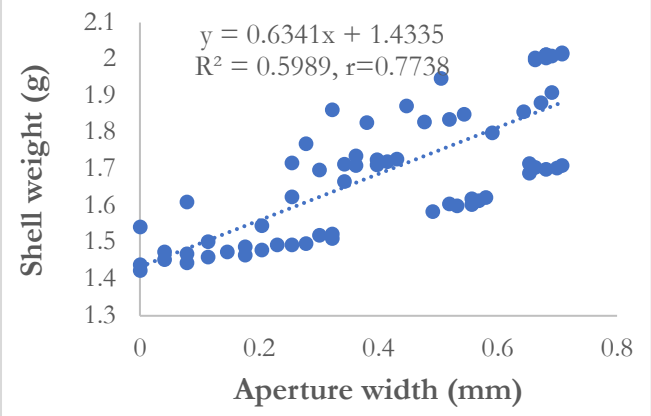

Figure 5. Log Aperture width – Log shell weight relationship of *Bulinus globusus* from River Wudill, Nigeria.

DNA yield and purity

DNA was eventually extracted from the total of 60 specimens of *B. globusus* and the quality of the DNA samples is shown in Figure6. Purity of DNA extracted from the analyzed samples ranged between 1.62 and 1.87. Mean DNA yield and purity were 120.03 ± 5.10 ng/µl and 1.81 ± 1.21. These values indicate that the samples were in pure condition without contamination of protein and RNA.

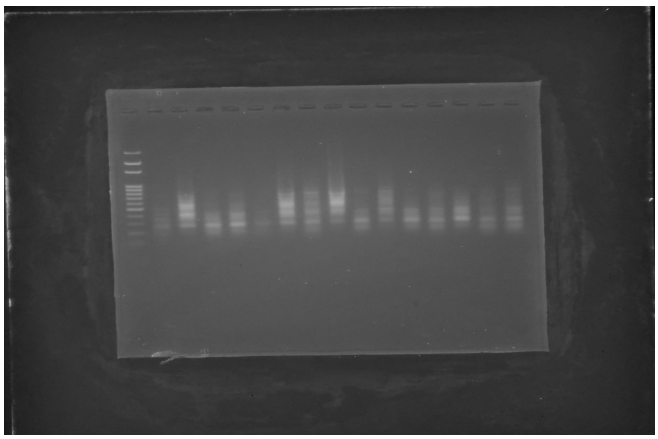

Figure 5. Gel profile of DNA samples of *Bulinus globusus* from River Wudill, Nigeria.

Discussion

In this study, the exponents were generally lower than 3, indicating negative allometric growth. The correlation coefficients (r) in all the shell variables were below "1', showing a week correlation between the parameters. Thus, indicating that the snail (*B. globusus*) body forms did not grow at the same proportion. This finding conforms to the report on the freshwater clam, *Egeria radiata* in the Forcados River, Niger Delta, Nigeria (Ehigiator and Osawaru, 2016).. Also, soft tissue yield of the snail is higher than 24 % reported for Swan mussel in Lake Chad (Bascinar *et al.*, 2009) and similar to the 37.7 % observed for *Unio stevenianus* (Çetinkaya, 1996). The growth of an individual snail could be limited due to the direct investment of energy to shell growth instead of the soft tissue (Ehigiator and Osawaru, 2016). This is due to the need for strong shell and a high capacity to live in adverse conditions.

The mean DNA yield and purity in this study indicate that the samples were in pure condition without contamination of protein and RNA, similar to previous reports on portunids and cichlids from Lagos Lagoon, Nigeria (Moruf *et al.*, 2019; Soyinka *et al.*, 2020). Samples of good quality are expected to

have absorbance ratio A260/A280 to lie in the range of 1.6 – 2.1 (Templeton *et al*., 2001). Furthermore, the DNA yield in this study is well above the minimum standard. The implication here is, despite the contaminants in some samples, the quality and quantity of the isolated genomic DNA is still very high and will give better and reliable results after been subjected to different downstream analyses.

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Conclusions

The fresh water snail, *Bulinus globusus* from River Wudil exhibits negative allometric growth pattern which is typical of many bivalve molluscs. About 33.7 % of the live weight of the snail species is made up of flesh by weight. The DNA yield in this study is well above the minimum standard and the pure extracts are good enough for further DNA analyses.

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