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Reproductive Biology of *Heterotis Niloticus* (Cuvier, 1829) in Alwero Reservoir, Gambella, Ethiopia

Ojuni Odier[1]*, Minwyelet Mingist[2], Gashaw Tesfaye[3] and Adamneh Dagne[3]

[1]*Department of Animal Production and health, College of Agriculture and Environmental science, Gambella University, Ethiopia*
[2]*School of fishery and wildlife, Department of Fishery and Aquatic Science, College of Agriculture and Environmental science, Bahir Dar University, Ethiopia*
[3]*Ethiopian institute of agriculture research national fishery and aquatic life research center, Ethiopia*

Abstract

This study was to investigate reproductive biology of *Heterotis niloticus* in Alwero Reservoir for better exploitation of fish resources which was conducted on monthly basis from December 2020 to November 2021 to know Length-weight relationships, sex ratios and size at first maturity of *H. niloticus*. Gillnets of 11 different mesh sizes (4, 6, 8, 10,12,14,16,18,20,22 and 24 cm) were used to sample the fishes. Total Length (TL) and total body weight (TW) of *H. niloticus* were measured to the nearest 0.1 cm and 1 g, respectively. The results of the study showed that the length-weight relationships for *H. niloticus* (TW = 10.502TL1.4; n =360; r2 = 0.749) and showed a curvilinear relationship, signifying the fishes growth were negative allometric growth, insignificant difference between sexes (P<0.05). The size at first sexual maturity (L50) for *H. niloticus* females 56.20 cm TL and 52.57 cm TL for males. H. niloticus have an extended breeding period from March to August, with peak spawning in June and July. The main intense in breeding activity occurred during the rainy months of April to August. The mean numbers of eggs per female were 19,698.5 eggs for *H. niloticus*, with total length range from 44 to 97 cm TL. Fecundity had a significant correlation (P<0.05) with both somatic weight and total length. Based on growth parameters indicated that the fish population is normal fish population with no apparent problems of over exploitation, although there is report in size reduction of fish by fishers. Unregulated open access resource use, illegal use of gillnets and uncoordinated water resources development activities existed.

Keywords: Alwero reservoir; Fecundity; Length-weight relationship; Sex ratio; Size at first Maturity

Introduction

Reproduction is a crucial stage in the life cycle of a fish that determine the demographic balance of a fish species [1]. It's associated with habitat differences, fish species type, differences in body condition and growth. *Heterotis niloticus* is a fish in the Osteoglossidae family. They distributed in White Nile system and Omo-Turkana in Ethiopia [2]. White Nile system includes Baro River, Alwero River, Gilo River, Akobo River, Pibor River and their tributaries and floodplain in Gambella regional state.

Heterotis niloticus has a high aquaculture potential due to its strong growth, its double breath, the good taste quality of its flesh and its relative high commercial value [3]. In the Alwero Reservoir, it is exploited from regularly by the artisanal fishery. *Heterotis niloticus* is the 2nd fish species commonly seen in the market next to tilapia [4].

Understanding of the breeding and fecundity of commercially important fishes is critical and can provide basic knowledge for the conservation and fish resources management. However, such knowledge is not recently available for the species in the Gambella water bodies in general and Alwero reservoir in particular; and this has hindered proper management of the fishery. Therefore, the objectives of this study was to determine breeding season, size at first maturity (L_{50}), length-weight relationship, condition factor, fecundity and sex ratio of *Heterotis niloticus* in Alwero reservoir with the aim of providing basic information for proper utilization and management of the stock.

Materials and Methods

Study site

Alwero reservoir is located in the Abobo Woreda (7052'19.2" N; 34029'56.4" E) at an altitude of 450 m, which is about 824 km Southeast of Addis Ababa (Figure 1). It has a surface area of about 74 km2 [5]. It has a tremendous resource regarding fish production that reaches an average of 144 tons of fish annually which created invaluable job opportunities for the surrounding community [6]. Three Sampling station, Kano, Gidip and Zero were selected purposively based on vegetation cover, human and animal interference intensity.

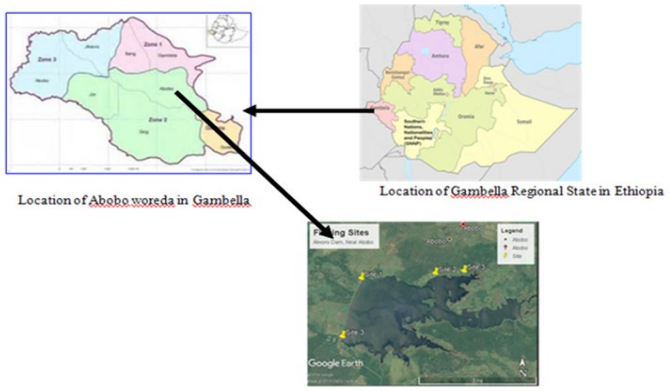

Figure 1: Location of Alwero reservoir in Gambella Regional State.

***Corresponding author:** Ojuni Odier, Department of Animal Production and health, College of Agriculture and Environmental science, Gambella University, Ethiopia, Tel: 251917834170; E-mail: agnewoj@gmail.com

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Sample collection and measurements

Experimental fishing was conducted monthly from December 2020 to November 2021 using multifilament gillnets of different stretched mesh sizes (4, 6, 8, 10, 12, 14, 16, 18, 20, 22 and 24 cm) made of 210 D/15 twines. Immediately after harvest, fishes were identified into species, Total Length (TL) for rounded and truncated caudal fine fishes (e.g. *O. niloticus* and *C. gariepinus*) and Fork Length (FL) for forked caudal fin fishes (e.g. B. docmac, C. carpio and L. intermedius) were measured to the nearest 0.1 cm and total weight to the nearest 0.1 g. Sexes of the harvested fishes were determined by inspecting visually and by dissecting the gonads for some biological parameter analysis. A five-point maturity scale was used to describe stages of gonads [7]; Immature - I; Developing - II; Maturing (and/or recovering for females) - III; Mature (and/or recovering for males) - IV; Ripe - V; Ripe running - VI; Spent (for females only)-VII. Males were graded into six stages because their gonads contained running milt most of the time and the spent condition was not distinct. The female gonads go through a well-defined stage of recovering. Recycling females of Stage III were designated as III R. The ovaries of *Heterotis niloticus* were split longitudinally and turned inside out, to ensure the penetration of the preservative before storing in labeled jars [8] and they were preserved in Gilson's fluid [9]. The preserved samples were then transported to the laboratories at Bahir Dar University College of Agriculture and Environmental Sciences for laboratory analysis.

Length-Weight Relationship and Condition Factor: The relationship between total length and total weight of the fishes were calculated using least squares regression analysis [8] as follows: $TW = aTL^b$, for total length. Where: TW-Total weight in grams, TL-Total length in centimeters, a - Intercept of the regression line and b-Slope of the regression line.

The condition or well-being of the species was determined by computing Fulton Condition Factor suggested by Bagenal and Tesch [8].

$$FCF = \left(\frac{TW}{TL^3}\right) x100$$, for Total length fish speciesd

Where: FCF - Fulton condition factor, TL-Total length in centimeters and TW-Total weight in grams

Estimation of Sex-Ratio: The numbers of female and male individuals were recorded for each sampling occasion. Sex- ratio (Female: Male) were then calculated for each fish species of the total sample. Chi-square test was employed to test if sex ratio varied from one-to-one in the total samples for each species as in Demeke Admassu (1994).

$$\text{Sex ratio} = \frac{\text{number of males}}{\text{number of females}}$$

Determination of Breeding Season: The breeding season of *Heterotis niloticus* were determined from monthly frequency of fish with ripe gonads and gonado-somatic index (GSI). The GSI for each fish was computed as the weight of the gonads in percent of total body weight and GSI were calculated using the formula employed by Marcano [10], Sarkar [11], and Gupta and Banerjee [12].

$$GSI = \left(\frac{\text{Weight of gonad}}{\text{Weight of fish}}\right) x100$$ Where, GSI - Gonado-somatic index

Estimation of Length at Fish Maturity (L_{50}): The average length at first maturity (L_{50}) has been defined as the length at which 50% of the individuals in a given length class reach maturity [13]. Thus, after classifying data by length class, the percentages of male and female *Heterotis niloticus* with mature gonads were plotted against length to estimate L_{50} graphically [14]. Thus, L_{m50} were estimated from the relationship between the percentages of mature fish (P) of length class (L) as described by the logistic function [15]:

$$p = e^{(\alpha+\beta)}/(1 + e^{-(\alpha+\beta L)})$$

and the value of L_{m50} was estimated using the following equation:

$$L_{m50} = -\alpha/\beta$$

Where, α and β was estimated using Marquardt's [16] algorithm of non-linear least squares regression.

Fecundity Estimation: Fecundity is the number of eggs produced per female per unit time (e.g., per spawning season). The fecundity of ripe gonads preserved in Gilson's fluid was estimated gravimetrically [17]. Thus, for each fish species, the numbers of eggs in three sub-samples of eggs each weighing 1 g, were determined from which average number of eggs per gram of ovary will be calculated. The weight of all the eggs in ovaries was also calculated. Fecundity as total number of eggs per ovary (F) was then estimated for each fish species as in:

F=W*n/w

Where, W=Weight of all eggs n=Average number of eggs per gram w=Weight of sub-samples of eggs (=1g)

In addition, relative fecundity as number of eggs per ovary weight as well as per total body weight was also calculated.

Data analysis

The length frequency distributions were used to describe the size structure of the fish, using the computer packages MS-Excel 2010 and SAS version 9. Mann-Whitney U test were used to analyze condition factor. Their maturity was identified by examining the gonads, and gonadal development will be staged in order to identify seasonal maturational cycles. The size at sexual transition was estimated by examining the size range in which males overlapped with females. The median value and its confidence limits were taken to represent the size at which sex change occurs. The timing of the spawning season was studied using the appearance of the gonads according to the predefined stages of maturation over time. Length-weight relationships were obtained using the log linear regression model $\log_{10}W_T = \log_{10}a + b\log_{10}L_T$ where W_T is the weight in grams, L_T is the total length in centimeters, log *a* is the intercept of the regression model, and *b* is the regression coefficient. The relationship between fecundity and some morphometric measurements (TL, TW and ovary weight) were determine using least squares regression. The size at sexual maturity (L_{50}) of females were estimated by fitting a logistic regression function, available from SAS version 9, to the proportion of mature fish in 5 cm size categories. The overall male to female sex ratio was calculated for the whole sample size at 5 cm class intervals. A two-sample independent-test will be used to determine the differences in size between females and males. For all statistical tests that were conducted, the level of significance will be set to $P < 0.05$.

Results

Length-weight relationship and condition factor

A total of 360 specimens from *Heterotis niloticus (n=360) in* Alwero reservoir were caught for the study of biological parameters using gillnets mesh sizes *(*4, 6, 8, 10,12,14,16,18,20,22 and 24 cm*)*. The total

length of *H. niloticus* range from 44-97 cm TL with their corresponding total weight range from 1400 to 9100 gm.

Length-weight relationships were determined for *Heterotis niloticus* in Alwero reservoir was curvilinear and statistically highly significant ($P < 0.05$). The line fitted to the data for *H. niloticus* (both sexes) described by the regression equations shown in Figures 2. An equation combined for fish ranging in length from 44 - 97 cm TL (*H. niloticus*), and the corresponding total weight from 1400 to 9100 gm.

Fulton Condition Factor (FCF) values (mean ± SE) of the *H. niloticus* were 2.44±0.08 for both sexes and for the separate sexes (2.42±0.11 for females and 2.47±0.11 for males). Average monthly Fulton's condition factor (K) of *H. niloticus* for both sexes were fitted and shown in Figures 3. The results were found to be significantly different between months (ANOVA, $P<0.05$). Lower values of k were observed during January and July for male and female respectively (Figure 3) which seems to match with the peak breeding season of the fish and decrease of water level. However, no significant difference was observed between sexes (ANOVA, $P > 0.05$).

Estimation of sex-ratio

Sex ratio of the *H. niloticus* is presented in Table 1. Females were more frequent than males in Alwero reservoir. The sex ratios of *H. niloticus* were insignificantly different from the hypothetical distribution of 1:1 ($P >0.05$) (Table 1).

Estimation of length at fish maturity (l_{50})

The L50 of fishes were found to be 56.20 cm TL and 52.57 cm TL for female and males *H. niloticus*, respectively (Figure 4). Based on the current study, on average males attain sexual maturity at relatively smaller size than females at Alwero reservoir.

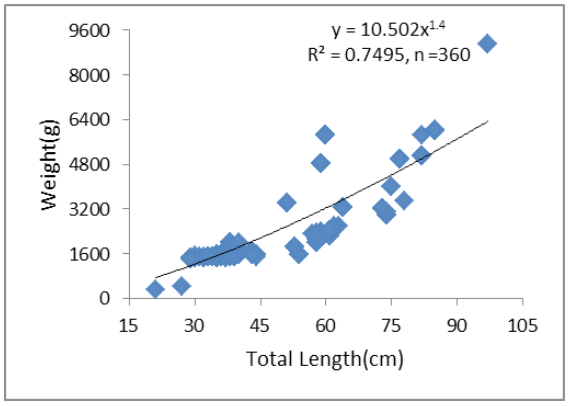

Figure 2: Length-weight relationship of *H. niloticus*.

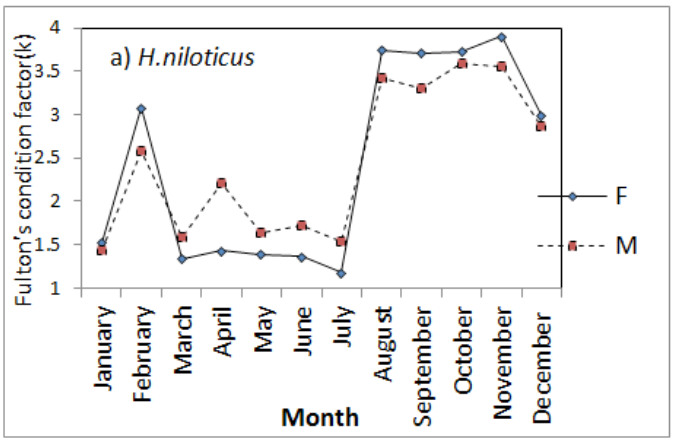

Figure 3: Fulton's condition factor (K) of *H. niloticus* in Alwero reservoir.

Table 1: Sex ratio of *H. niloticus*, *G. niloticus, B. bajad* and *O. niloticus* at Alwero reservoir.

Fish species	Total	Females	Males	F:M	Chi-square
H. niloticus	360	192	168	1:1.14	0.2059

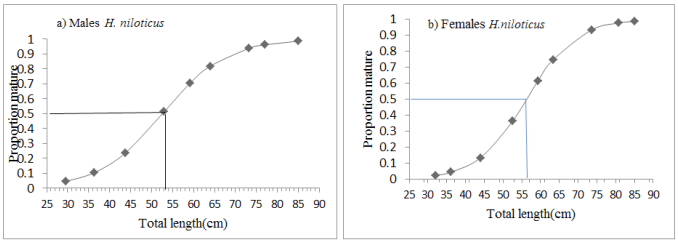

Figure 4: The proportion in different length groups of males (a) and females (b) of *H. niloticus* from the breeding seasons in Alwero reservoir.

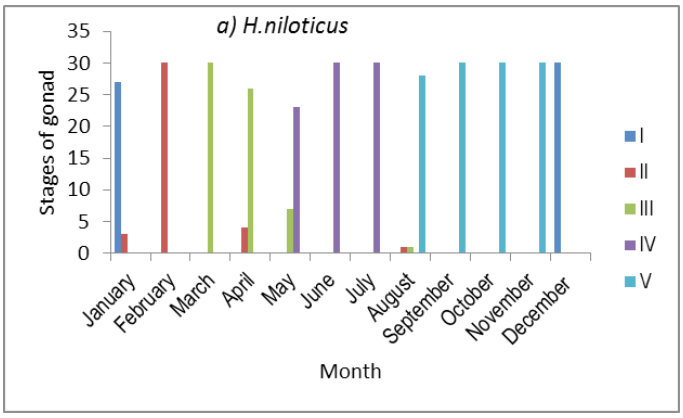

Figure 5: Breeding season of a) *H. niloticus* in Alwero reservoir.

Determination of breeding season

The breeding season of *H. niloticus* were determined from percentages of fish with ripe gonads taken monthly from December 2020 to November 2021. Fish that had immature gonads (Stages I and II) were found in January, February, April and August for *H. niloticus*. Fish that were still developing (Stage III) occurred in March to May and peak March. Fully matured gonads (Stage IV) were observed from May to July and peak in June for *H. niloticus*. Fish with spent gonads (Stage V) were found in August to November, August to October and July to October for *H. niloticus* (Figure 5). The gonadosomatic index (GSI) for females *H. niloticus* ranged between 0.61% and 2.40% with a mean of 1.50% ± 0.05%.

Fecundity estimation

Fecundity of *H. niloticus* was range between 10,430 to 28,967 eggs with average of 19,698.5 eggs, with correspondent total length range from 44 to 97 cm TL. Fecundity had a significant correlation ($P<0.05$) with both somatic weight and total length for *H. niloticus* in Alwero reservoir. The relationship between fecundity and total length, total weight and gonad weight were shown in Fig.6. Fecundity showed an increase with the increase in size of the fish and ovary weight (Figure 6).

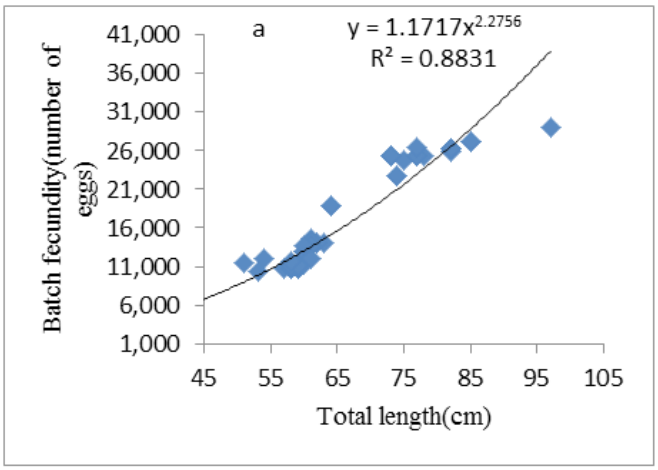

Figure 6: Relationship between fecundity and Total length (a) *H. niloticus* in Alwero reservoir.

Discussion

Length-weight relationship and condition factor

The relationship between Total length and total weight for *H. niloticus* in Alwero reservoir was curvilinear and statically significant (Figures 1). This agrees with other works in tropical lakes and reservoirs [18]. The regression coefficient of *H. niloticus* in this study is (b=1.39) which show negative allometric growth in *Alwero reservoir* unlike positive allometric growth Patten report by Gatriay Tut [19] in Gilo River (b=3.03) and wetlands (b= 3.58) for both seasons in Gambella region. Similarly, disagree with the finding of Ezekiel and Abowei, [20] in Amassoma flood plain (b=3.17). However, these findings agree with report of Olanrewaju [21] in Lake Alau (b=1.80) that indicate negative allometric growth.

The Fulton's condition factor (K) indicates the relative plumpness or degree of well-being of the fish. The average K value of *H. niloticus* (2.44±0.08) in Alwero reservoir is higher than values reported for the same fish species *H. niloticus* (0.75 ± 0.16 and 0.79 ± 0.19) from Gilo river and wetlands respectively in Gambella region [19]. Generally high mean condition factor for *H. niloticus* indicates good condition of fishes throughout the study period at Alwero reservoir. Agree with Pauker and Rogers, [22] that indicate higher body condition is correlated with high energy content, adequate food availability, and reproductive potential and favourable environmental conditions.

Average monthly Fulton's condition factor (K) of *H. niloticus* fish species for both sexes were fitted and shown in Figures 2. The results were found to be significantly different between months (ANOVA, $P<0.05$). Lower values of k were observed during January and July for male and female *H. niloticus* respectively (Figure 2) which seems to match with the peak breeding season of the fish and decrease of water level. However, no significant difference was observed between sexes (ANOVA, $P > 0.05$).

Estimation of Sex-Ratio

The overall sex ratio of *H. niloticus* was not significantly different from 1:1 ($P > 0.05$) in the present study. Female dominant sex ratios (1: 1.17; X2= 0.20593; $p > 0.05$) were seen for *H. niloticus* at Alwero Reservoir (Table 1). This agrees with the results obtained for the same species in Agneby River [23]. However, male dominant sex ratios over female *H. niloticus* were reported in Anambra River, Nigeria [24]; and in Lake Hlan and So^ River [25]. The unequal sex ratio found in the present study for *H. niloticus* was probably attributable to behavioural differences between the sexes (growth rate, differences maturity rates, mortality rates, or migratory patterns) between the sexes which might have made females more vulnerable to be caught in passive or active (chest & traps) gears like gill nets. According to Matsuyama [26] unequal sex ratios between the male and female sexes could be caused by other biological mechanisms like differences maturity rates, mortality rates, or migratory patterns of fish. In addition, preponderance of females has been attributed to sexual separation during spawning activity, gear type and fishing site for O. niloticus in Lake Hawassa [27]. Further study is required to see if the same factors could be responsible for sex ratio in the Alwero reservoir fish species.

Determination of breeding season

The smallest male found with ripe gonads was 44cm TL and 1582 g., while the smallest female in breeding condition was 51 cm TL and weighed 3430 g for *H. niloticus*. The extended breeding period of 5 to 6 months (March to July for *H. niloticus*) could be a strategy to ensure that their offspring reach a relatively bigger size that places them in a better position to predate on fry and fingerlings of other fish species that would breed later in the year. Extended intense breeding seasons (May to August) for *H. niloticus* were documented by [25] in the So^ River-floodplain system. Generally the breeding of most tropical fishes occurred during rainy season when physicochemical conditions of the aquatic environment are favourable [28, 29], although continuous all year round breeding has been reported in some tropical fish species like O.niloticus in Fincha reservoir [30] which is similar trend at Alwero resevoir. Fully matured gonads were observed from May to July and peak in June and July for *H. niloticus*. This finding partially agreed with the work of Kouakou [23] for *H. niloticus* which stated that fully matured gonads were observed from June to august and from November to December.

Estimation of Length at Fish Maturity (L_{50})

The information of knowing sexual maturity is useful to identify sizes that fish are capable to produce, optimum age of first capture of fish species, time and place of spawning. The sizes at 50% maturity of *H. niloticus* in current study were 56.20 cm for females and 52.27 cm for males which were higher than those reported by Kouakou [23] for the species in Agneby River (43.63 cm for males and 48 cm for females). Nearly similar with finding of Adite [25] stated that the size at which 50% of individuals were mature was about 57.5 cm TL for both sexes of *H. niloticus* from the So^ River–Lake Hlan system.

Fecundity estimation

The fecundity recorded in this study varied between 10,430 to 28,967 eggs with average of 19,698.5 eggs for *H. niloticus* suggesting that the species has medium fecundity. This finding in agreement with the report of Kouakou [23] who recorded an average of 15,507.5 of eggs for *H. niloticus*. High parental care fish species are exhibit low fecundity characterizes [31]. The significant correlation ($P<0.05$) of fecundity with both somatic weight and Total length suggests that fecundity increases with increase in weight and length of *H. niloticus* at Alwero reservoir. According to King, [32] the number of eggs produced increased as the body weight of fish increased, whereas Shinkafi [33] reported insignificant correlation in the length and fecundity.

Conclusions and Recommendations

The aim of this study was to investigate the reproductive biology

of *H. niloticus* for better exploitation of resources in Alwero Reservoir. This study revealed that the length-weight relationship of *H. niloticus* was curvilinear and statistically significant, showed a negative allometric growth which implies that the fish species they tend to become thinner as they grow larger. High mean condition factor recoded for *H. niloticus* indicates good condition of fishes throughout the study period at Alwero reservoir. It was found to be different between months but not between the sexes. *H. niloticus* in Alwero Reservoir followed the general pattern of breeding behavior in tropical water bodies were relatively higher proportions of fish were in breeding condition during the onset of the rainy season when there is high flooding. Females were more frequent than males, males *H. niloticus* matured at smaller size than female in Alwero reservoir. Indeed capture size of the stock should be determined taking into account the L50 of females, which may otherwise remove the spawning fish during their peak breeding season. However, we recommend the fishers have to use gillnets greater than 12 cm stretched mesh size for *H. niloticus* to ensure sustainable utilization of the stock in Alwero reservoir. A closed season is recommended during the peak breeding period for *H. niloticus*. The results of this study have therefore provided baseline information which could enhance production potential of this fish species and its sustainable development and management in Alwero Reservoir.

Declaration

Author's contribution statement Ojuni Odier, Minwyelet Mingist, Gashaw Tesfaye and Adamneh Dagne conceived and designed the experiments; performed the experiments; analyzed and interpreted the data; contributed reagents, materials, analysis tools; and wrote the paper.

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Data availability statement

The data that has been used is confidential.

Declaration of interested statement

The authors declare that no conflict of interests.

Additional information

No additional information is available for this paper.

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