Evaluating Morphological Asymmetry in Three Species of the Genus *Hydrocynus* from the White Nile in Sudan

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**Abstract** The present study aimed to determine and evaluate the level of fluctuating asymmetry (FA) of morphological characters for three species of *Hydrocynus* from the White Nile. In *H. vittatus* 41% of morphological characters fluctuated to the right side and 59% to left side. In *H. brevis* 50% fluctuated to right and 50% to left side, while in *H. forskalii* 34% fluctuated to right and 66% fluctuated to left side. More scales were recorded along the lateral line on the right side of *H. vittatus* and *H. brevis* compared to *H. forskalii*, while more gill rakers were recorded on the left side of *H. vittatus*. The smallest asymmetry index was found for PVFL of the three species and the largest index was found for POL of *H. brevis*. Mean asymmetry index was (3.5%) in *H. forskalii*, (5.1%) and (4.9%) in *H. vittatus* and *H. brevis*, respectively. Absolute asymmetry values were significantly correlated with character size for the number of lateral line scales, head length, pectoral fin length and ventral fin length (P<0.05) and highly correlated for snout length and caudal peduncle length (P<0.001) in the three species. Correlations between means of bilateral characters and body size were significant (P<0.05) for the head length, pelvic fin length and pectoral spine length of the three species. This study is the first of its kind in species of the Nile fish. The present result can be a base line for further study of fluctuating asymmetry in fish and as indicators to detect the level of pollutants in the Nile and the condition of the environment and the organisms.

**Keywords** Fluctuating asymmetry; *Hydrocynus*; Meristic; Morphometric; Sudan; White Nile

1 Background
The level of fluctuating asymmetry (FA) is most often used to estimate developmental instability of individuals and populations of fish, when bilateral traits show some variations in the size or counts of the two sides 'left-right asymmetry', where phenotypic traits of left or right individuals differ asymmetrically (Moller, 1997). When environmental stressors of the environment affects the total morphology of the organisms, the overall symmetry and developmental stability will be disturbed (Daloso, 2014). Many zoologists studying fish morphology may regard fish bodies as being laterally symmetric. However, Palmer and Strobeck (1986) showed that there is a high index of fluctuating asymmetry in various fish populations under strong environmental pressure. Hence, it can give rise to decreased developmental stability of individuals, which may result in reduced performance of fitness components (Clarke, 1995; Moller and Swaddle, 1997). Asymmetry in fishes was first described in scale-eating cichlids from Lake Tanganyika, in the form of bilateral dimorphism when opening their mouths (Mboko et al., 1998). Recently, however, asymmetry of morphological traits has been documented (Almeida et al., 2008; Lutterschmidt et al., 2016). Bilateral asymmetry, in individual and population levels of fish, was found to relate positively to a wide range of abiotic, biotic and genetic stresses (Allenbach et al., 1999; Franco et al., 2002; Estes et al., 2006), and could be sensitive to different levels of individual density in captive conditions (Leary et al., 1991) or increases under genetic stresses such as hybridization, inbreeding and loss of genetic variation (Mazzi et al., 2002; Dongen, 2006), particularly in reared fishes (Palma et al., 2001; Fessehaye et al., 2007).

*Hydrocynus vittatus* (Castelnau, 1861), *H. brevis* (Cuvier and Valenciennes, 1849) and *H. forskalii* (Cuvier, 1819) are three species of the Nile fishes in the family Alestidae. These species are abundant in varied freshwater environments, including the White Nile, Blue Nile and the main Nile and Lake Nubia, but *H. forskalii* is the commonest species (Bailey, 1994). The synonymy of *H. forskalii* with *H. vittatus* was revised by Brewster (1986). However, while examining samples of fish for taxonomic revision of the three species (Elagba and Wigdan, 2015),
surprisingly we noticed some alteration in external morphology and level of ‘left-right asymmetry’, where some morphological characters (metric and meristic) of left and right sides differed asymmetrically in some specimens. These observations prompted us to measure and evaluate the morphological asymmetry (Fluctuating asymmetry, FA) in the three species of Hydrocynus. The aim of the present study is to determine and evaluate the level of FA of morphological characters (9 metric and 5 meristic) of the three species of Hydrocynus. This study is the first of its kind in species of the Nile fish. It is an attempt to explain the causes and relationship of this morphological asymmetry with the environmental stress caused by different environmental conditions in the Nile. The result of the study is expected to be a base line for further study of fluctuating asymmetry in fish and can be employed to detect the level of pollutants in the Nile environment, in evaluating the condition of the environment and the organisms and as a potential indicator for describing developmental instability of the Nile fishes.

2 Materials and Methods

2.1 Fish collection
The study was carried out for thirty specimens of Hydrocynus species, were obtained from the White Nile at Jebel Aula Dam, 45 Km South of Khartoum. Comparative material included 10 from each of H. vittatus, H. brevis and H. forskalii. Each individual specimen was identified according to the original description of Boulenger (1907). The institutional abbreviations followed Daget and Grosse (1984).

2.2 Morphological characters
Nine paired bilateral morphometric characters (Length of the head (HL), eye diameter (ED), post-orbital length (POL), length of the snout (SNL), length of pectoral fin (PFL), ventral fin (VFL), pelvic fin (PVFL), pectoral spine (PSL) and caudal peduncle (CPL); and five meristic (number of pectoral fin rays (PFR), number of scales along the lateral line (LLS), scale rows above (DLS) and below lateral line (VLS), and number of gill rakers (GR) were used to estimate fluctuating asymmetry of the three species of Hydrocynus, following Holcik et al. (1989). Metric characters were measured to the nearest 0.01 mm, using a fine dial caliper. Every character was estimated as mean of three repeated measures of each variable. Meristic characters were chosen due to their functional importance as sensory (lateral line), structural (scale rows), locomotive (fins) and feeding (gill rakers) traits.

2.3 Fluctuating asymmetry (FA) analysis
Character size was calculated as the average between both sides [(R+L)/2]. Measure of individual fluctuating asymmetry (FA) was calculated as the signed mean differences between left and right measures and estimated using the formula of Palmer and Strobeck (1986): (FA = Ri−Li), where (Ri) is right side and (Li) left side of morphological character. Because metric characters and FA values may be positively influenced by body size (Pertoldi and Kristensen, 2015), the relationship between each metric character and body size was analyzed by performing regressions of FA against standard body length. The magnitude of absolute FA was assessed as the unsigned difference between the measurements of the right and left sides (| R−L |). Spearman rank and Pearson correlations were performed between absolute values of FA for meristic and morphometric characters, respectively and character size. An index of asymmetry was calculated as follows (R−L/R) %, according to (Rossi et al., 2003). For individuals with R>L their right sides dominated over the left, and the index was assigned a positive value. In contrast, for individuals with R<L the index was assigned a negative index value.

3 Results
The results of asymmetry analysis for the measured morphological characters of the population of H. vittatus (1), H. brevis (2) and H. forskalii (3) showed that most investigated individuals of the three species expressed same degree of asymmetry as shown in (Figure 1; Figure 2). The magnitude of fluctuating asymmetry was different in the three species although H. forskalii showed more tendency towards the left side. The snout of all specimens of the three species was longer on the left side compared to right side, indicating fluctuation to the left side “lefty”. More scales were recorded along the lateral line on the right side of H. vittatus and H. brevis compared to H. forskalii, while more gill rakers were also recorded on the left side of H. vittatus compared to the right one. The smallest asymmetry index (0.1 to 0.4%) was found for PVFL of the three species and the largest index (4.5%) was found for POL of H. brevis (Table 1). Although the three species had almost same means of characters size, mean
asymmetry index was lower in *H. forskalii* (3.5%) compared to (5.1%) and (4.9%) in *H. vittatus* and *H. brevis*, respectively. In *H. vittatus* 41% of then measured morphological characters fluctuated to the right side and 59% to left side. In *H. brevis* 50% fluctuated to the right side and 50% to left side, while in *H. forskalii* 34% fluctuated to the right and 66% fluctuated to the left side.

Absolute asymmetry values were significantly correlated with character size for the number of lateral line scales, head length, pectoral fin length and ventral fin length (Spearman correlation, P>0.05) and highly correlated for snout length and caudal peduncle length ( Pearson correlation, P>0.001) in the three species. By Spearman and Pearson correlations between means of bilateral characters and standard length, we found pelvic fin length ($r^2 = 0.7$), head length ($r^2 = 0.6$), pectoral spine length ($r^2 = 0.6$), snout length ($r^2 = 0.5$) and ventral fin length ($r^2 = 0.5$) of the three species to be positively influenced by standard length, P < 0.05.

Figure 1 Mean asymmetry index (R-L/R) % of metric characters for individuals of *H. vittatus* (1), *H. brevis* (2) and *H. forskalii* (3)

Figure 2 Mean asymmetry index (R-L/R) % of meristic characters for individuals of *H. vittatus* (1), *H. brevis* (2) and *H. forskalii* (3)
Table 1 Mean of character size and the asymmetry indexes of H. vittatus (1), H. brevis (2) and H. forskalii (3)

| Character | Character size (R+L)/2 | Asymmetry index (R-L/R)% |
|-----------|------------------------|--------------------------|
|           | Fish 1 | Fish 2 | Fish 3 | Fish 1 | Fish 2 | Fish 3 |
| HL        | 6.5    | 6.3    | 6.5    | 0.6    | 0.6    | 0.6    |
| ED        | 2.1    | 2      | 2.2    | 0.4    | 2.4    | 0.9    |
| POL       | 3.4    | 3.3    | 3.8    | 0.6    | 4.5    | 0.8    |
| SNL       | 2.8    | 2.6    | 2.9    | 10.7   | 9.5    | 5.8    |
| PFL       | 1.8    | 1.8    | 1.9    | 2.7    | 0      | 1.1    |
| VFL       | 1.7    | 1.6    | 1.8    | 1      | 2.5    | 5.7    |
| PVFL      | 14.7   | 11.7   | 13     | 0.1    | 0.2    | 0.4    |
| PSL       | 4.6    | 4.3    | 4.6    | 0.2    | 1.9    | 1.2    |
| CPL       | 3      | 2.9    | 3.2    | 1.2    | 1.8    | 0.6    |
| PFR       | 14.1   | 13.45  | 12.05  | 4.4    | 0.8    | 2.5    |
| LLS       | 57.1   | 55.6   | 54.3   | 2.1    | 2.2    | 1.7    |
| DLS       | 9.8    | 10.5   | 11.3   | 3.8    | 5.5    | 4.1    |
| VLS       | 6.5    | 5.7    | 6.2    | 7.8    | 4.3    | 0.3    |
| GR        | 55.4   | 54.9   | 56.6   | 2.5    | 0.8    | 0.8    |
| Mean      | 24.5   | 23.6   | 24.1   | 5.1    | 4.9    | 3.5    |

4 Discussion

In this study significant difference in the degree of FA among the populations of the three species was detected. Occurrence of FA in a single individual may prove meaningful biologically in identifying environmental stressors within the Nile water. The Different degrees of FA among individuals of fish in each population may identify environmental instability. Individual specimens sampled could have experienced different developmental conditions during their development. Differences in the percentage of FA may be attributed to the ability of the traits to buffer developmental alterations (Lens et al., 2002). Therefore, environmental condition takes part for the overall condition of the species and may enhance its fitness to resist alterations. According to Allan (2004), Richards et al. (1996) and Roy et al. (2003), human activities along the Nile can have some impacts on water quality, habitat, and aquatic biota and can play a significant role in defining Nile condition and thus create developmental stressors resulting in FA. In addition, pesticides and fertilizers are main factors to contamination of water and aquatic natural resources (Manjare et al., 2010). However, water chemistry may have a more direct influence on FA and may be a more powerful predictor of developmental stress. Other factors such as nutrition, chemical contaminants and pH have also been shown to increase FA (Allenbach, 2011). Annual variability in temperature and water levels could also impact a variety of stressors contributing to increase variability among individuals. Many studies have shown FA to be a reliable bio-indicator of environmental stress (Hardersen, 2000; Seixas et al., 2016).

Since, asymmetry in the morphology of fish specimens was observed, it might be a sign that fishes in the sampling area underneath environmental disturbances. Observed fluctuating asymmetry would be an indication of existing pollutants within the fish habitat which consequently affected its morphology. The continuity of the fishes in the polluted and disturbed environment will likely affect fish morphology and cause asymmetry. The present data shows the indication for FA of the fishes that can be a result of a stressed environment probably from different types of aquatic pollutants. The polluted ecosystem will eventually cause morphological variation as these effluents interfere during its growth and development (Bonada and Williams, 2002).

Many Studies showed that greater fluctuating asymmetry is the outcome of the species towards environmental situation (Ducos and Tubago, 2015). Other studies have also showed direct relationship between environmental stress and the increase of abnormalities (Taylor, 2001; Lemly, 2002; Hermita et al., 2013; Lutterschmidt et al.,
2016) and parasites in fishes (Schwaiger, 2001; Almeida et al., 2008). Tull and Brussard (2006) also found FA in the western fence lizard (Sceloporus occidentalis) to be greater among individuals exposed to off-highway vehicle disturbance, and Lajus et al. (2003) found FA in some characters of eelpout (Zoarces viviparous) to correlate with environmental conditions of salinity and temperature. Additionally Estes et al. (2006) found eastern mosquitofish (Gambusia holbrooki) from a stream with high levels of paper mill effluent to have greater FA than mosquitofish from streams free of effluent. Zakeyudin et al. (2012) investigated the spotted barb (Puntius binotatus) and used it as an essential bio-marker to environmental stress or the health condition of the aquatic habitat. Cabuga et al. (2017) also reported that the approach of FA and physico-chemical parameters is significant for evaluating environmental condition as well as species state of well-being. However, the present results of FA and our interpretation can serve as the foundational data for investigating the effects of pollution on the aquatic ecosystem and biodiversity of the Nile. Future investigation of FA within the Nile system can be focused on environmental water quality, microhabitat assessment, and changes in hydrology which may be more meaningful for predicting responses in FA. Species with greater responses in FA that could better serve as biological indicators of the health of a given habitat.

Authors’ contributions
Analysis of data and writing of manuscript were done by Dr. Elagba Mohamed while morphological measurements and counts were done by Wigdan Al-Awadi. All authors read and approved the final manuscript.

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