Fluctuating asymmetry as a measure of developmental stability of three-spotted gourami, *Trichopodus trichopterus* (Pallas, 1770) in Lake Sebu, South Cotabato, Philippines

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Abstract. Studies concerning environmental changes have become the focus of today's researches as the environment exhibit stress due to various, extreme natural and anthropogenic factors. These disruptions largely affect the development of organisms which play an important role as bioindicators to determine the present conditioin of the area they inhabit. Fluctuations in their body shapes (fluctuating asymmetry) are dependent to their adaptability or their capacity to buffer stress posed on their habitats. Hence, the inability of these species to develop perfect symmetry can be associated to the disturbances in their environment. This study aimed to determine the developmental stability of the three-spotted gourami, *Trichopodus trichopterus*, in Lake Sebu, South Cotabato, Philippines. 30 male and 30 female *T. trichopterus* individuals were subjected to Landmark-based geometric morphometrics. Procrustes ANOVA was employed for the assessment of FA. Results showed fluctuations within females, while males reflected no variations. The fluctuations on the body shape structures among *T. trichopterus* females have proven to be more susceptible in the environmental stress than the males due to the various functions in their courtship, maneuverability and reproduction roles. It has been identified that the separation between their sexes implicated the sexual dimorphism in their body shapes. Moreover, the results manifested a high FA in the body shapes of *T. trichopterus* implying a high level of stress in the water systems of the lake.

Key Words: fluctuations, morphology, bioindicator, Gourami, sexual dimorphism.

Introduction. Developmental stability is the ability of an organism to buffer environmental and genetic perturbations experienced during its ontogeny and assumed to be influenced by genotype, environment and/or genotype by environment (Waddington 1942; Zakharov 1989; Clarke 1998; Van Dongen & Lens 2000; Ducos & Tabugo 2015). Inability to maintain precise development creates variations in morphological characters between the right and left sides of an individual manifesting directionally random, subtle deviation from perfect symmetry, referred to as fluctuating asymmetry (FA) (Palmer & Strobeck 1986; Palmer 1994). Developmental instability is commonly measured using FA and is believed to be occurring from various exogenous and endogenous stresses collectively referred to as developmental noise or perturbations (Reimchem & Nosil 2001; Ducos & Tabugo 2015). Generally, high trait FA is associated with these perturbations and stresses during the development of an individual fish, and along with individuals and populations that develop in stressful and or marginal environment (Hermita et al 2013). Hence, as the level of environmental stress or instability increases, so is the level of fluctuating asymmetry (Antuaco & Leyesa 2004). It is for these reasons, other than being inexpensive and easy to carry out, that FA has received increasing attention in the last decade among a variety of existing bioindicators (Leung et al 2000).

Applied biologists are interested in monitoring environmental stress, preferably before stress irreversibly damages populations using changes in biota, i.e. bioindicators, to indicate these stresses (Clarke 1993; Bunn 1995; Leung et al 2000). Fish have been widely documented as useful indicators of environmental water quality because of their differential susceptibility to pollution (Niagaga et al 2011) and has an advantage over other organisms present in rivers and lakes because they are relatively long-lived and mobile (Karr et al 1986).

Three-spotted gourami (*Trichopodus trichopterus*), locally known as "gourami", is among the common fish species found in Lake Sebu, along with Nile tilapia (*Oreochromis niloticus*), trimac (*Amphilophus trimaculatus*), silver perch (*Leiopotherapon plumbeus*), guppy (*Poecilia reticulata*) and celebes goby (*Glossogobius celebius*) and is contributing to the municipality's economic impact particularly in commerce and tourism. Lake Sebu is one of the best tourist spots in the Philippines, as such, dubbed as the "summer capital of Southern Mindanao" recognized for its amazing view and seeming nature. Its economy is generally based on aquaculture which has caused rather adverse effects to the lake's health due to overexploitation as a consequence of the lack of management strategies. This could trigger a phenomenon locally referred to as "*kamahong*" leading to massive fish kills.

Monitoring the current environmental status of the lake is necessary. Several fish species have been studied to assess the condition and water quality of Lake Sebu (Natividad et al 2015; Paña et al 2015; Lecera et al 2015; Lacorte et al 2015). However, less attention have been given to *T. trichopterus* as indicators of environmental condition. *T. trichopterus* is exceedingly resistant and easily adapts to tremendous environmental conditions due to its eminent features and superior adaptability. Noteworthy, *T. trichopterus* species can survive cases of hypoxia, a reduction in the concentration of dissolved oxygen in an aquatic environment through its auxiliary respiratory structure which allows it to breathe gaseous oxygen in a hypoxic solution. The apparent disturbance in Lake Sebu has probably caused stress on these species. This amount of stressors affects the degree of fluctuations in the development of the *T. trichopterus*, considering they have to compensate stress by requiring energy consequently affecting growth, developmental precision, and reproduction (Khoen & Bayne 1989; Sommer 1996; Leung et al 2000), which predefines the ecological health of its habitat making it an ideal subject for the present study.

Hence, the study aims to determine the developmental stability of *T. trichopterus* from Lake Sebu using FA analyses. As a locally common species in the area, the species may serve as a biological indicator. In doing so, the study would provide beneficial data as basis in developing management schemes for lake conservation.

Material and Method

Study area. Lake Sebu is located in the southwestern part of South Cotabato province, situated between 6°13′0″N - 124°42′0″E (Figure 1). The lake is greatly compromised by the presence of fish cages where overfeeding and overstocking of fishes is employed by local fish farmers, aggravated by domestic wastes and effluents from communities and commercial establishments in the vicinity of the lake.

Figure 1. Map of the Philippines showing South Cotabato located in Mindanao (lower right) and an aerial view of Lake Sebu (upper right).

Fluctuating asymmetry of T. trichopterus

Fish specimen identification and separation by sex. A total of 60 individuals composed of 30 males and 30 females were collected using customary fish-netting technique. Samples were placed in a Styrofoam box filled with ice to preserve the color and body shape. Species were identified based on www.fishbase.org. Males and females were identified based on external morphology of the *T. trichopterus*, particularly on the dorsal fin. Male *T. trichopterus* has longer and more pointed dorsal fin while females have shorter and round dorsal fin (Figure 2).

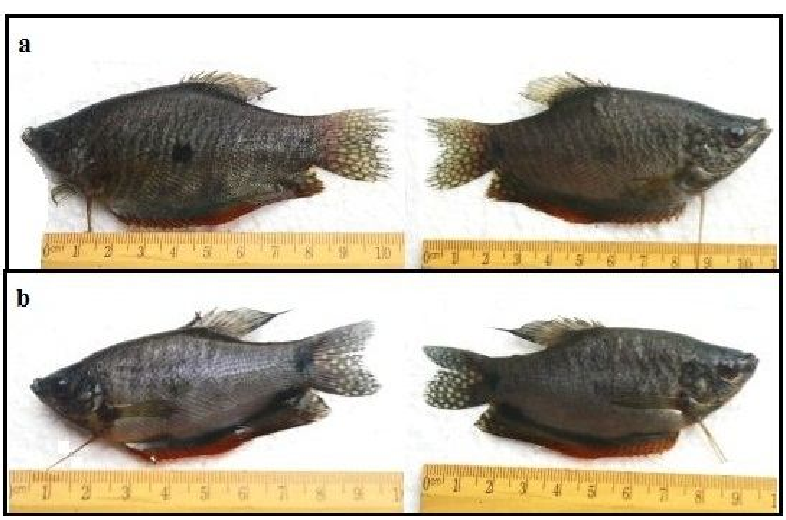

Figure 2. Left and right side of *T. trichopterus*: (a) Female; (b) Male (original).

Digitization of fish specimen and landmarking. Digital imaging of fish samples was done using Sony digicam (14 megapixels). Both left and right lateral sides were taken. Captured images were then converted to TPS format files using tpsDig version 2.10 program. Analyses of landmarks were obtained using Thin-Plate Spline (TPS) series to incorporate curving features within the image. A total of 20 landmark points (equivalent to 20 X and 20 Y Cartesian coordinates) were selected following landmarks used by Dorado et al (2012) to best represent the external shape of the body. Location and description of landmark points is shown in Figure 3.

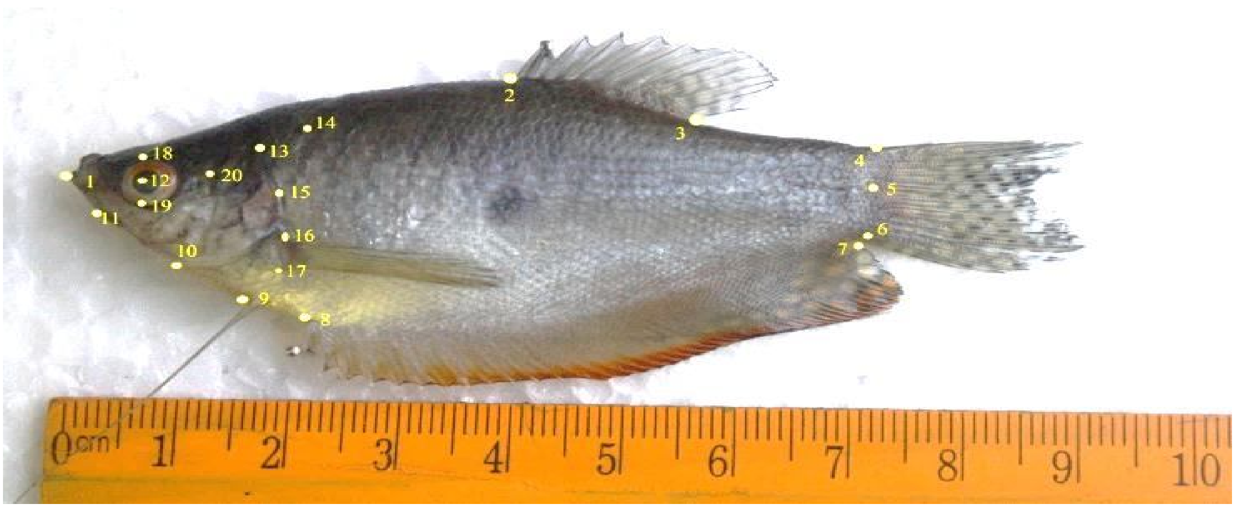

Figure 3. Landmarks used for digitizing image of Three-spotted Gourami, *Trichopodus trichopterus*: (1) snout tip; (2) & (3) anterior and posterior insertion of the dorsal fin; (4) & (6) points of maximum curvature of the peduncle; (5) posterior body extremity (7) & (8) posterior and anterior insertion of the anal fin; (9) insertion of the pelvic fin; (10) insertion of the operculum at the lateral profile; (11) posterior extremity of premaxillar; (12) center of the eye; (13) superior insertion of operculum; (14) beginning of the lateral line; (15) point of maximum extension of operculum on the lateral profile; (16) & (17) superior and inferior insertion of the pectoral fin; (18) & (19) superior and inferior margin of the eye; (20) superior margin of the pre-operculum (original).

Shape analysis. FA of *T. trichopterus* were determined using the generated X and Y coordinates. The coordinates data taken from both lateral sides of the fishes were subjected to Symmetry and Asymmetry in Geometric Data (SAGE) software (version 1.04, Marquez 2007). Proctuses ANOVA were employed to calculate and quantify the asymmetry between samples.

Result and Discussion. Table 1 shows the calculated fluctuation in body shape of *T. trichopterus* species using Proctuses ANOVA. Statistical results clearly reveal a high phenotypic variation among the specimens collected. It is observed that the individual symmetry of shape of the left-right orientation, the variations on the left and right sides of both sexes on the side and individual X sides scores showed a highly significant value (0.000). This signifies a manifestation of fluctuating asymmetry in *T. trichopterus species* in Lake Sebu.

Table 1

Procrustes ANOVA analysis for male Trichopodus trichopterus

Category	*SS*	*Df*	*MS*	*F*	*p-values*
Individuals	0.1539	1044	0.0001	2.966	0
(Sides) Directional asymmetry	0.0581	36	0.0016	32.4464	0
Fluctuating asymmetry	0.0519	1044	0	4.3434	0
Measurement error	0.0494	4320	0	-	-

Endurable ranges of most fishes to various parameters dropped due to the factors impacting the water quality of the lake. Very low levels of dissolved oxygen and high level of coliform have contributed much to the recent fish kills in the area. Moreover, the decaying organic materials in the lake which are consumed by bacteria added up to severe dispersal of oxygen content in the lake water, and the moderate increase in temperature aided to bacterial growth. Another factor is due to overstocking of fishes in a cage that exceeds beyond its capacity. The larger the number of individuals in a cage, the smaller the ability of a fish to accommodate its needs such as food and oxygen. Difference in the levels of individual density in captive condition resulted to variations in body shapes on both lateral sides of the individual (Leary et al 1991). Since the body shape of an organism is a result of its response to the environment they inhabit (Russo et al 2007), the genotype of an organism may change with the environmental conditions over time (Iwamoto et al 2012).

The analysis of the departures from left-right body shapes of FA are considered to result from stress during development (Nosil & Reimchen 2001; Russo et al 2007) and these departures from symmetry indicate disruption of normal development most likely due to genetic or environmental stresses (Parsons 1990). Since the analysis of shape is a fundamental part of biological research (Adams et al 2004), female *T. trichopterus* was found to have significant FA and affected by developmental instability as data shown below.

Table 2

Procrustes ANOVA analysis for female *Trichopodus trichopterus*

Category	*Ss*	*Df*	*Ms*	*F*	*P-value*	*Remarks*
Individual (shape/symmetry variation)	0.4278	1044	0.0004	1.3691	0	Not significant
Sides (directional asymmetry)	0.0531	36	0.0015	4.9302	0	Not significant
Individual x sides (fluctuating asymmetry)	0.3125	1044	0.0003	1.1667	0.0006	Significant
Measurement error	1.1082	4320	4320	-	-	-

To further support the data, PCA analysis comparing male and female *T. trichopterus* is performed with percentage interaction and affected landmarks (Table 3).

As shown in Table 3, the overall percentage interaction (FA interaction) in male *T. trichopterus* is 71.1%, lesser than the total 85.2839% in females from the 5% effective principal components. Affected landmark in PCA1 in males are revealed in landmarks 1, 2, 8, 10, 11, 12, 15, 18, 19, and 20, whereas all landmarks are affected in females. PCA2 in the affected landmarks in males showed in anterior insertion in the anal fin (8), insertion of the operculum at the lateral profile (10), posterior extremity of premaxillar (11), and in the inferior margin of the eye. In contrast, PCA2 in females revealed fluctuations mostly in anterior insertion of the dorsal fin (2), anterior insertion of the anal fin (8), insertion of the operculum at the lateral profile (10), posterior extremity of premaxillar (11), center of the eye (12), superior insertion of operculum (13), and beginning of the lateral line (14) and the point of maximum extension of operculum on the lateral profile (15). However, only males exhibit PCA3-5 with landmarks affected in: anterior & posterior insertion of the dorsal fin (2 & 3), anterior insertion of the anal fin (8), insertion of the operculum at the lateral profile (10), posterior extremity of premaxiallar (11), superior insertion of operculum (13), beginning of the lateral line (14), and point of maximum extension of operculum on the lateral profile (15).

Table 3

Upper 5% Principal Component Analysis (PCA) of FA in male and female *Trichopodus trichopterus*

Principle Component Analysis	*Male*		*Female*	
	Affected landmarks	*% Interaction male*	*Affected landmarks*	*% Interaction female*
PC_1	1, 2 8, 10, 11, 12, 15, 18, 19, 20	29.2198%	1,2,3,4,5,6,7,8,9,10,11,12, 13,14,15,16,17,18,19,20	78.0555%
PC_2	8, 10, 11, 19	15.0817%	2, 8, 10, 11, 12, 13,14, 15	7.2284%
PC_3	3, 10, 11, 13, 14, 15	11.5822%	-	-
PC_4	2, 13, 14	7.99%	-	-
PC_5	3, 8, 10	6.6656%	-	-
Overall %	-	71.1%	-	85.2839%

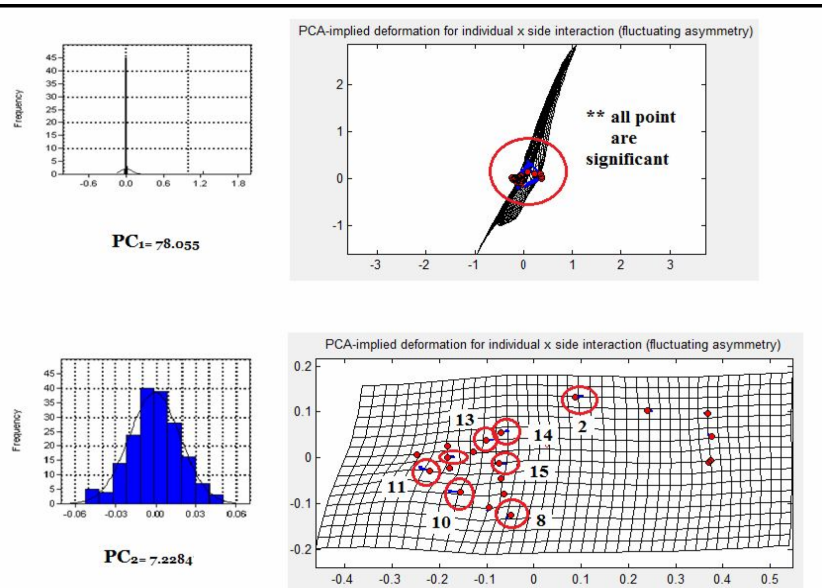

Figure 4. Principal Components Analysis (PCA) in female *Trichopodus trichopterus.* This indicates the deformation of FA with its histogram, basing the frequency on the fluctuation. The percentage suggested the proportion of variation for which the relevant components described. Encircled points referred to the affected landmark.

Figure 4a and 4b shows the shape variation patterns of covariation in the positions of landmarks in the body of both sexes of *T. trichopterus*.

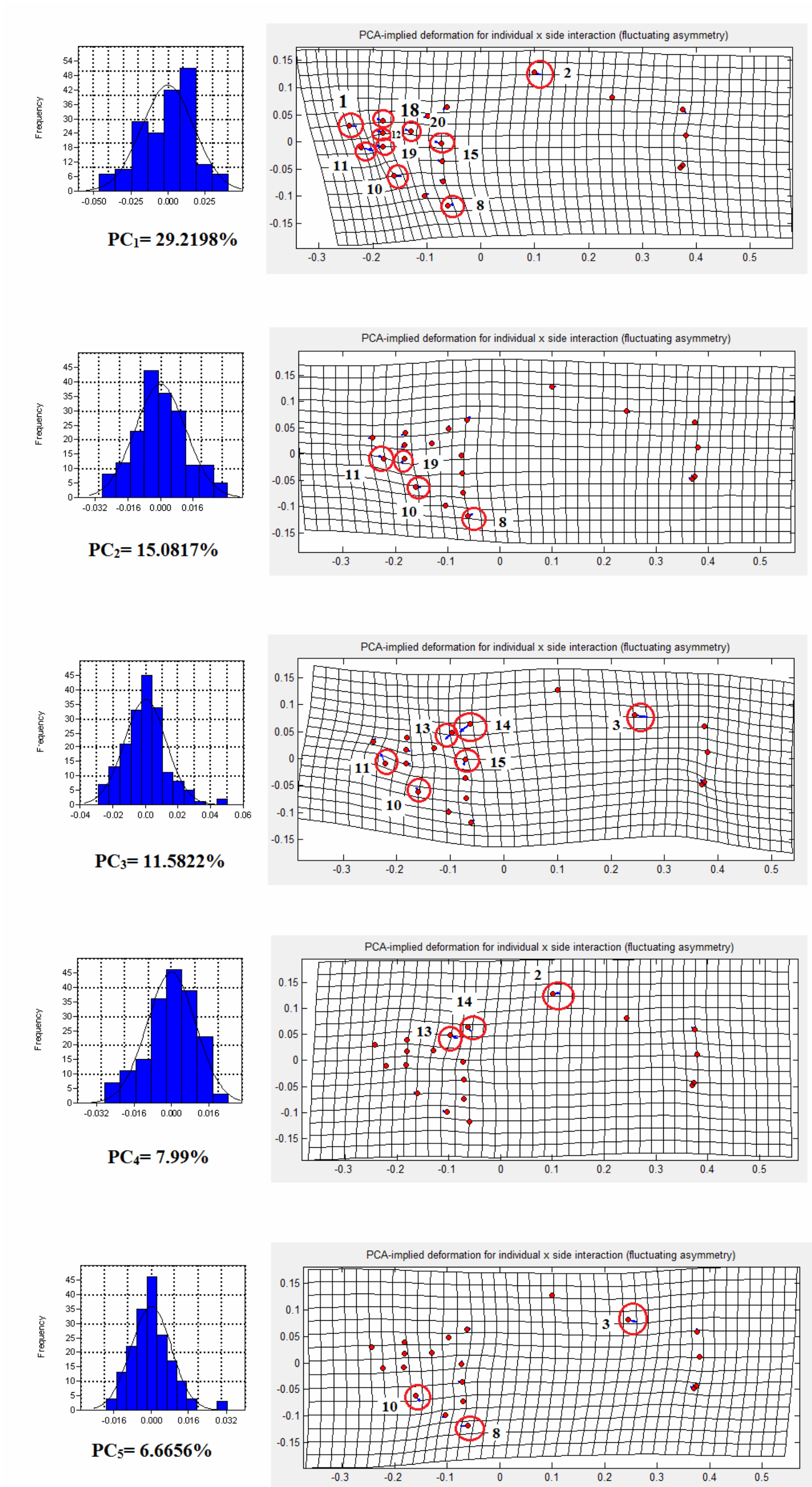

Figure 5. Principal Components Analysis (PCA) in male *Trichopodus trichopterus.* This indicates the deformation of FA with its histogram, basing the frequency on the fluctuation. The percentage suggested the proportion of variation for which the relevant components described. Encircled points referred to the affected landmark.

Table 4

PCA implied deformation of individual x side interaction (FA) with Histogram of *Trichopodus trichopterus*

Landmark points	Character	Male	Female
1	Snout tip	√	√
2	Anterior insertion of dorsal fin	√	√√
3	Exterior insertion of dorsal fin	√	√
4	Points of maximum curvature of the peduncle		√
5	Posterior body extremity		√
6	Points of maximum curvature of the peduncle		√
7	Posterior insertion of the anal fin		√
8	Anterior insertion of the anal fin	√√	√√
9	Insertion of the pelvic fin		√
10	Insertion of the operculum at the lateral profile	√√	√√
11	Posterior extremity of premaxillar	√√	√√
12	Center of the eye	√	√√
13	Superior insertion of operculum	√	√√
14	Beginning of the lateral line	√	√√
15	Point of maximum extension of operculum on the lateral profile	√	√
16	Superior insertion of the pectoral fin		√
17	Inferior insertion of the pectoral fin		√
18	Superior margin of the eye	√	√
19	Inferior margin of the eye	√√	√
20	Superior margin of the preoperculum	√	√
	Total	17	20

Check (√) in the table signifies the affected landmarks and the affected regions. First Two PCA test for the presence of traits asymmetry is presented. One check (√) implies that there is present at least in a PCAs, Double check (√√) also signifies a presence in the first two PCA.

Conclusions. Differences in the body shape of the two sexes of *T. trichopterus* have concluded to have been affected from the various functions of their courtship, maneuverability and reproductive roles. Male have longer, more pointed dorsal and anal fins, and is known for being aggressive and dominant in their territory. Its slim body played an important role to its maneuverability and efficiency to protect the eggs from potential predators. Conversely, female tend to have greater posterior dorsal and caudal region curvatures which support their balance and maneuverability in the water, and along with broader belly which become rounded for laying eggs. Females are heavier than males. Thus, the differences between sexes have greater impact on their developmental stability. Moreover, the significant differences in the body shapes of the three-spotted gourami showed that females exhibit more significant variations in its morphology than male specimens. Therefore, female *T. trichopterus* are more susceptible in environmental stress, since it has also been observed in some studies that the differences in the patterns of trait FA between sexes might mean that there might exists differences in the levels of developmental homeostasis between males and females. Male *T. trichopterus* have mechanisms that buffer developmental stress, making it capable of maintaining homeostasis whereas female lack the said mechanism.

Overall, this study showed the significant application of FA in the body shapes of *T. trichopterus,* in which played an important role in the disturbed lakes in Lake Sebu. This would provide beneficial data as basis for locals to enhance the condition and to develop management schemes for lake conservation.

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