ORIGINAL ARTICLES

Sexual Dimorphism in Tilapia, *Oreochromis mossambicus* (Peters, 1852) from Lake Lanao, Philippines

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ABSTRACT

The major objective of this study is to determine shape dimorphism between sexes of the introduced species of the tilapia *O. mossambicus* from Lake Lanao Philippines, using landmark-based geometric morphometrics. A total of 84 specimens (41 females and 43 males) were analyzed. Eighteen landmark points were digitized on the specimens from whom relative warp scores were derived. Discriminant function analysis and thin-plate spline plot showed significant difference between body shape of the sexes with males exhibiting bigger head, along with elongated dorsal and anal fin bases. The elongated dorsal and anal fins could be a product of ecological selection.

Introduction

Sexual shape dimorphism has traditionally been ascribed to sexual selection. The concept of sexual dimorphism predicts that differences in the reproductive roles between sexes may influence patterns of selection and could lead to sex differences in morphological attributes such as the shape of its body (Casselman, S.J. and A.I. Schulte-Hostedde, 2004). Alternatively, sexual dimorphism can also evolve by ecological selection acting differentially on the sexes and thus favouring both dimorphic niches and, as a consequence, dimorphic trophic structures (Hedrick A.V. and E.J. Temeles. 1989; Herler, J. *et al*, 2010). Ecological factors may provide additional selective pressures for shape differences between sexes, thus these traits may be functional adaptations to illustrate different ecological niches between sexes (Spoljaric M.A. and T.E. Reimchen, 2008).

Oreochromis mossambicus is one of of the 77 species of fish all belonging to family Cichlidae, popularly known as " tilapia" (George, T.T., 2006). Which originated exclusively from the African continent and Palestine (Philippart, J.C. and J.C. Ruwet, 1982). The various species have been introduced and cultured as food species in many countries because of its many desirable traits for aquaculture. In the Philippines it is becoming the most popular freshwater fish(De Silva, Sena, 2003; Gutiérrez, A.T. and J.K. Reaser, 2005) and is now considered a widespread species in Lake Lanao, a lake in southern Philippines, where it was introduced in the 1960s(Rosagaron, R.P., 2001.

Three species of tilapia are commonly introduced, *O. mossambicus*, *O. aureus* and *O. niloticus*. In the Philippines, *O. mossambicus* (introduced in 1950), and *O. niloticus* (introduced in 1972) are most commonly used in aquaculture, with most farmers preferring *O. niloticus* because of its faster growth and lighter color (Guerrero, R.D., III. 1985). Farmers however, from Lake Lanao and Lake Sebu, two lakes from southern Philippines, are known to prefer the taste of *O. mossambicus* over *O. Niloticus* (Oliva, L.P., 1983), hence stocking their farms with the former or a combination of both species. Laboratory bred individuals are known to show signs of sexual maturity at standard length of 4-7 cm (Oliveira, R.F. and V.C. Almada, 1995). whereas it was also recorded that *T. mossambica* collected from Lake Sibaya, South Africa reach sexual maturity at a standard length of 8 cm for females and 10 cm for males (Trewavas, E., 1982).

Sexually dimorphic traits in tilapia are well established. These traits are often used in aquaculture to separate the sexes. It was noted that mature males have thicker lip in upper jaw(Oliveira, R.F. and V.C. Almada, 1995). It was also established that in the *Oreochromis* spp. males are larger than females and exhibits conspicuous breeding colors (Trewavas, E., 1982). While in *Oreochromis* (formerly Tilapia) *aureus* and *O. Galilaeus*, males can be distinguished from females by the number of of openings in their genital papilla (two for males, three for females) and the more pointed shape of the anal fin in males which is rounded in females (Brzeski, V.J. and R.W. Doyle, 1988). Two sets of traits were identified in *O. mossambicus* that tend to accelerate in males: jaw structure and height of dorsal and anal fins which could be used for sexing individuals (Oliveira, R.F. and V.C. Almada, 1995).

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Many of these studies measured sexual dimorphism in tilapia using either laboratory stock specimens reared in aquaria or large tanks((Oliveira, R.F. and V.C. Almada, 1995; Chervinski, J., 1965). or were collected from aquaculture ponds. It is known however, that the environment in which the organisms live can affect their morphology and has been shown as a force in modelling the morphology of fishes. In fact fishes of the family Cichlidae have long been known for their spectacular adaptive radiation and phenotypic plasticity(Klingenberg, C.P., *et al.*, 2003). The principal differences in some cichlids among them are on their mouth and orbital diameter and are related to their trophic ecology (Daud, D.K., *et al.*, 2003). It was also observed that specimens from large lakes exhibit increased sexual shape dimorphism and decreased in small and shallow ponds (Spoljaric M.A. and T.E. Reimchen, 2008).

Thus this study re-examines sexual dimorphism of *O. mossambicus* using specimens collected from the natural environment of Lake Lanao, using the new method of landmark-based geometric morphometrics. One major advantage of this approach over traditional morphometrics is that the result of the analyses could be visualized as configurations of landmarks back in the original space of the organism and could be represented as actual shapes or shape deformations rather than only as statistical scatter plots (Adams, D.C., *et al.*, 2002).

Materials And Methods:

Sampling and Study Area. Lake Lanao (8^0 00' N, 123^0 50' E) is the largest (357 km^2) lake in Mindanao, Philippines with an altitude of 702 m, a mean depth of 60.3 m and an altitude of 702 m. Five major river systems drain into the lake from the surrounding watershed areas, while Agus River is the one and only river draining the Lake on its north side, towards Iligan Bay. Fresh specimens were bought from fishermen fishing for wild populations of tilapia in the northern side of the Lake near Marawi City (Fig. 1) in the morning of October 15, 2009. The fresh specimens were transported inside a styropore box and immediately brought to the laboratory for processing. The specimens range in size from 10-12 cm in standard length.
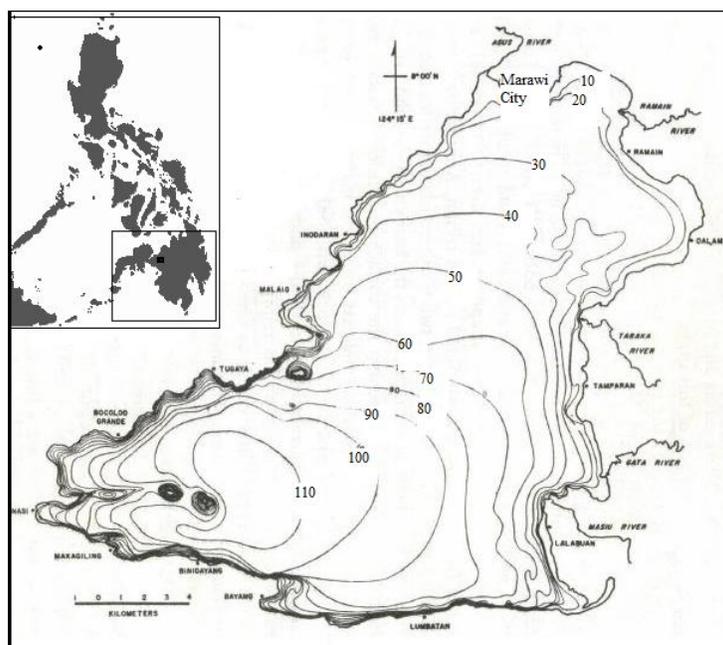

Fig. 1: Bathymetric map of Lake Lanao (after Frey, 1974). Contour interval is 10 m below the mean lake water level. It shows that the lake is shallowest on the northern side, becoming progressively deeper going south. It is being drained   to the north by way of Agus River towards Iligan Bay. (inset) Map of the Philippines showing the approximate location of Lake Lanao (black box) in the island of Mindanao (boxed).

Processing and Digitization of the Fish Specimens. Digital images from the left side of the samples were prepared from these selected specimens. These images were produced by a flat-bed scanner at a resolution of 600

dpi. From among 100 of the images, 84 (41 females and 43 males) were chosen for their excellent quality and included in this study. Sexing by examination of the gonads was done after scanning.

Eighteen landmarks (equivalent to 18 X and 18 Y Cartesian coordinates) were digitized on the images using the TpsDig (version 2.12) (Fig. 2)[20]. These landmarks were selected to provide a comprehensive summary of the morphology of the fish specimens. Digitization was done in tri-replicates for each fish sample.
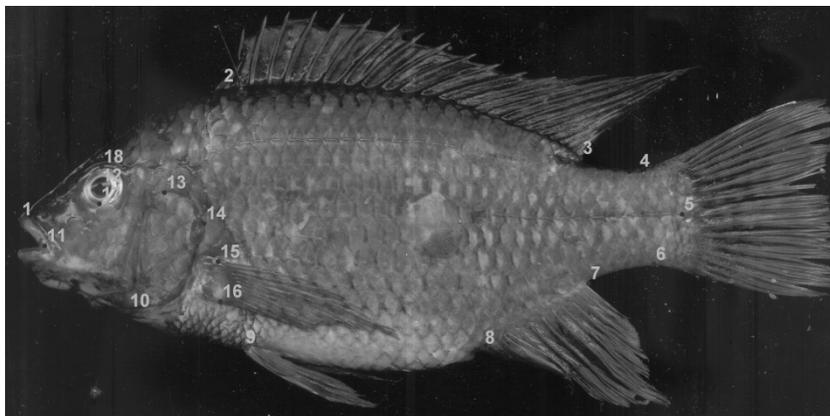

Fig. 2: A scanned image of *O. mossambicus*, digitized with the 18 landmarks as follows: (1) snout tip; (2) and (3) anterior and posterior insertion of the dorsal fin; (4) and (6) dorsal and ventral region of the caudal peduncle where there is the greatest curvature; (5) posteriormost body extremity; (7) and (8) posterior and anterior insertion of the anal fin; (9) insertion of the pelvic fin; (10) insertion of the operculum on the lateral profile; (11) posterior extremity of premaxillar; (12) centre of the eye; (13) superior insertion of operculum; (14) point of maximum extension of operculum on the lateral profile; (15 and 16) superior and inferior insertion of the pectoral fin; (17) and (18) superior and inferior margin of the eye.

Shape Analysis. The quantified data from the landmark points that were digitized on the specimens contain both shape and non-shape components of variation[19]. Since this study is focused on shape analysis, the non-shape components need to be removed before shape analysis could proceed. These non-shaped components were removed by General Procrustes Analysis (GPA) using TpsRelw ver. 1.36 (Rohlf, F.J., 2007). GPA aligned all the specimens in morphospace, eliminating size and rotational/translational differences. It proceeded as follows: first, the landmark specimens were translated to a common centroid at the origin of the reference coordinate system   at point (X=0, Y=0). Then the set of landmark coordinates of each fish sample were scaled to unit centroid size thereby removing size differences and permitting analysis of body shape. Finally, the landmark configurations of all fishes were rotated to minimize the sum of squared distances between corresponding landmarks (Bookstein, F.L., 1991). This step removed residual translational and rotational differences in the way the fishes were oriented in the flat-bed scanner when the digital images were taken.

Using the thin-plate spline equation and the standard formula for uniform shape components, a weight matrix (containing uniform and non-uniform shape components) from the aligned specimens were generated (Bookstein, F.L. 1991). Variability in body shapes was then examined via relative warp (RW) analysis of the set of uniform and non-uniform components of shape using TpsRelw ver. 1.36 generating new variables in the form RW scores (Rohlf, F.J., 2007). The RW scores were then subjected to Discriminant Function Analysis (DFA) to test for differences of body shape between sexes using the PAST software (PAleontological STatistics, version 1.27 (Hammer, O., *et al.,* 2001). In DFA the relative warp scores were treated as independent variables and a multivariate function was defined such that males and females were maximally discriminated.

To visualize the differences between body shapes of the sexes, the software Tps Spline version 1.20 was used(Rohlf, F.J., 2004). It produces transformation grids that show the shape change from a grid with square cells superimposed onto the average landmark configuration to a grid that is deformed to fit a target configuration. This comparison is based on Procrustees distance (d^2), which is the standard measure for the magnitude of shape differences used in geometric morphometrics (Bookstein, F.L., 1991).

Results And Discussion

Patterns of shape variation of the species is shown and is summarized via frequency histograms of the RW scores together with the grid plots of the landmark points showing the extremes of shape variation along the first six RW axes (Fig. 3) which explain 75 % of the total shape variation. These variations occur in the head region (lengthening and broadening) and the body region (elongation and shortening of the fin bases) as shown in the plots representing the extremes of the six relative warps.
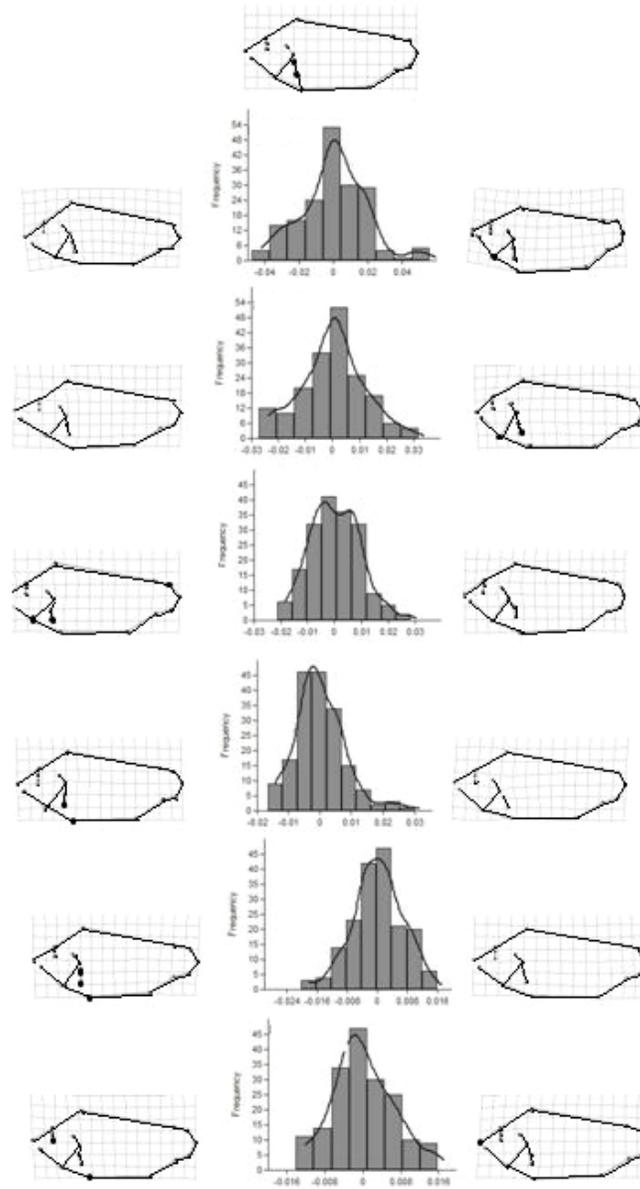

Fig. 3. Summary of geometric morphometric analysis of body shape among *O. mossambicus*. It shows the frequency histograms of each of the relative warp axis, and the extremes of their variation in body shape (plots to the left and right of the histograms) produced by the first six relative warps explaining 75% of the total total shape variation (RW1 = 36.83%, RW2 = 13.8%, RW3 = 8.75%, RW4 = 6.40%, RW5 = 4.68%, RW6 = 4.13%). The uppermost plot is the consensus morphology.

Much of these variations can be explained by sexual dimorphism. Figure 4 is the DFA of the relative warp scores between sexes, which shows that body shape of male *O. mossambicus* is different from that of the female (p<<0.05).
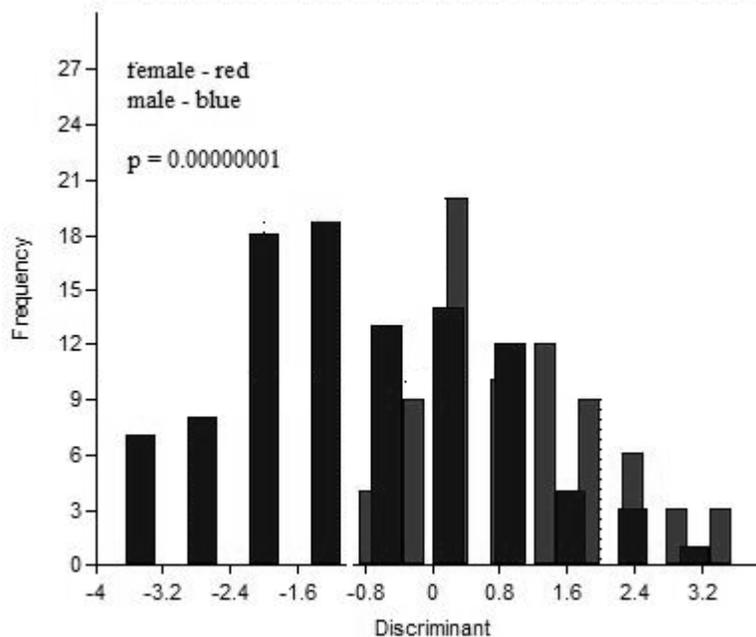

Fig. 4. DFA graph of the frequency and distribution of relative warp scores of body shape variation between male and female of *O. mossambicus* with the difference between sexes being highly significant (p << 0.05).

The visible pattern of major body shape variation differentiating the sexes is shown in Tps Spline plot (Fig. 5), and occurs in two sets: (1) those of the head region, and (2) those of the trunk/abdominal region. In the head region, the male, is with elongated jaw and pre-orbital region (expansion of the grids between landmarks 1 and 11 , and between landmarks 1 and 12) longer head (expansion of the grids between landmark 1 and 14) and deeper head (expansion of grids between landmarks 10 and 17). In the trunk region, the male is shown with longer dorsal fin base (expansion of the grids between landmarks 2 and 3), longer anal fin base (expansion of the grids between landmarks 7 and 8), and longer abdominal region for the females (expansion of the grids between landmarks 8 and 9).
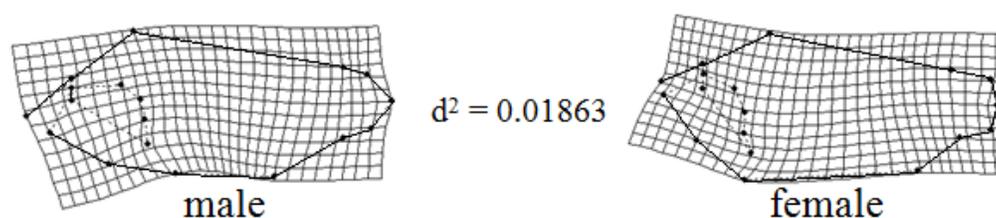

$$d^2 = 0.01863$$

Fig. 5. Thin Plate Spline plot of both sexes of *O. mossambicus* plotted against each other (exaggerated five-fold for better visualization of the difference). It shows the males have a deeper and longer head with the bases of the dorsal and anal fins being longer. The ventral abdominal region (between the insertion of the pelvic fin and anal fin) is also more elongated in females.

The bigger head measurements observe in males in this study is in agreement with result on hybrid specimens (*O. mossambicus* x *O. urolepis*), wher body depth was at the level of the eye and at the level of the dorsal fin together with premaxilla width to discrimate the sexes (Brzeski, V.J. and Doyle, R.W., 1988). Studies on *O. mossambicus*, also identified the jaw structure as one set of characters whose growth accelerates in males and together with the height of dorsal and anal fins was attributed to both intra- and intersexual selection (Oliveira, R.F. and V.C. Almada, 1995).

The second set of characters shown in this study to exhibit differences between the sexes involve the dorsal and anal fins whose bases are more elongated, (from the anterior point of attachment to the posterior-most point of

attachment) in males, was not reported in previous studies. The result of these fin elongations in males would be an increase in the span of the fins from the leading edge to the tip of the trailing edge and a change in its aspect ratio. These fin elongations can be argued as triggered by sexual selection, as it can be effective in agonistic behaviour to threaten rivals. A characteristic of this behaviour is broadside displays with full erection of the fins (Brutton, M.N. and R.E. Boltt., 1975; Baerends, G.P. and J.M. Baerends-van Roon, 1950; Neil, E.H. 1966).

The fact however, remains that fins exist first as functional structures enabling fishes to manoeuvre in an aquatic medium. Any change therefore in its form and total surface area would first impact on the fish capacity to manoeuvre. The natural selection pressure therefore, for the fins, is towards a fish becoming more efficient on its preferred behaviour and habit while conserving energy to enhance its fitness. Any other function that maybe ascribed to these fins should be treated as secondary and may either accentuate or attenuate such traits as shown in studies on fruit flies (Brutton, M.N. and R.E. Boltt., 1975; Baerends, G.P. and J.M. Baerends-van Roon, 1950; Neil, E.H. 1966).

The dorsal and anal fins in fishes have been reported to act as stabilizers by inhibiting roll movements during steady swimming and turning, and at the same time to contribute to thrust production by the caudal fin (Standen, E.M. and G.V. Lauder, 2005; Lauder, G.V. and E.G. Drucker, 2004; Drucker, E.G. and G.V. Lauder, 2002). The elongation of these fins, as observed in *O. mossambicus* of the present study, would then increase the fin's total surface area, imparting on the sexes differing capabilities to manoeuvre. This anal and fin difference between them may have been promoted by their differing preferred habits. The same could be argued on the more pointed trailing edge of the dorsal and anal fins in the males of some tilapias species (*Tilapia galilaea*, and *T. nilotica*), and the increased height of both the dorsal and anal fins from specimens in culture ponds or aquaria (Oliveira, R.F. and V.C. Almada, 1995; Chervinski, J., 1965).

In the natural environment of Lake Lanao, habitats are certainly more complex than artificial impoundments and would offer wild populations a variety of environments to accommodate any inclination towards a different habit and could promote the observed present result. Perhaps a bit of evidence to support the above contention is the result of the study on *O. mossambicus* from Lake Sibaya of South Africa (Brutton, M.N., R.E. Boltt., 1975). The sexes were reported to differ in their distribution with the approach of the warm season, with larger females usually being confined to the deeper regions, while small and larger males are abundant in shallower regions, and later in the season the females becoming more abundant in the shallows than males.

Conclusion:

This study was conducted to determine body shape dimorphism in *O. mossambicus* from the natural environment of Lake Lanao using Landmark-based Geometric Morphometrics coupled with multivariate statistical analysis. Results showed enlargement of the head and its associated structures in males, which conforms to the results of previous studies. The elongation of the base of the dorsal and anal fins however were previously unreported and could be attributed to ecological selection as a result of a more complex environment of the Lake. A further study on the sexual dimorphism of the same species from a confined less complex environment using the same method of geometric morphometrics and comparing the result to the present study, would be interesting.

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