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Effect of temperature and feed rate on the body shape of *Oreochromis shiranus*, a widely-cultured tilapia species in Malawi

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ABSTRACT

Considering the vitalness of traits such as body shape and size in influencing the marketability of fish, geometric morphometrics was utilized to explore if raising fish at different temperatures and feedrates would affect such important traits. MANOVA revealed significant differences in body shape among fish raised at different temperatures but not those fed at different levels of their body weight. In general, the fish that were raised at 20°C, regardless of the feedrate, had shorter but deeper bodies, larger eyes and longer heads as opposed to the longer and slender bodies, smaller eyes and shorter heads found in those reared at 25°C and 30°C. On size, ANOVA revealed significant differences along temperature and feedrate gradients in a way that fish reared at 20°C were the smallest, followed by those at 25°C and then the largest were those at 30°C. On feedrate, it was only at 30°C where fish fed at 6% body weight were significantly larger in size than those fed at 3% body weight. These findings are discussed in terms of best management practices, especially on how to combine such two abiotic factors in order to maximize growth within the shortest period possible, considering the economical implications.

Keywords: Fry, Mahalanobis Distance, Centroid Size, Canonical Variate Analysis, Poikilotherms

INTRODUCTION

Aquaculture production in Malawi is still very low and hinges on very few tilapia species (contributing about 93% of total production), namely: *Tilapia rendalli*, *Oreochromis karongae* and *O. shiranus*, and one catifsh species, *Clarias gariepinus*. Of the abiotic factors that constrain tilapia production in Malawi and other African countries, temperature features highly as this varies from place to place and season to season throughout the year. Considering that fish are poikilotherms, water temperature is a critical factor since it influences several biological processes, including survival, feed utilization and in turn growth. Physiological processes that control growth, survival and reproduction in fish bear a positive relationship with water temperature (Atwood *et al*., 2003). Water temperature interacts with other management practices such as stocking density and feed composition to affect growth of tilapias (Musuka, 2006). Due to the high gut evacuation rate, tilapias need to be fed frequently in order to achieve the best growth rate (Malcom & Brendan 2000). This, however, depends on both water temperature and fish size. The feed must be digested and assimilated so as to ensure high economic returns, therefore it is necessary to use an optimum feeding level. It is therefore necessary to optimize these rearing conditions, i.e. temperature and feeding levels, due to their direct bearing on the growth performance of fish, in order to boost aquaculture production. Several researchers have investigated how fish form is affected by abiotic factors like temperature, salinity etc. (see Hubbs, 1926; Barlow, 1961).

Fish form encompasses both size and shape. Unfortunately in aquaculture, it is only size that is considered when one wants to explore the effect of any treatment on the growth of fish. It is common knowledge that growth of fish, or any organism, is not just unidirectional but rather multidimensional, hence this approach of exploring growth through only standard length in fish leaves certain vital information of fish form unexplored, i.e. shape. Therefore the aim of this study is to utilize geometric morphometrics to uncover the effect of temperature and feed rate on the shape and size of widely cultured *O. shiranus*, and thus make geometric morphometrics known to Malawian aquaculturists who can add it to their tool kit when it comes to fish quality assessment.

MATERIAL AND METHODS

Source of fish and experimental set up

Fingerlings of *Oreochromis shiranus* were collected from an earthen pond at Bunda College fish farm using seine net, graded according to weight. Thereafter, the fish were transferred to a 2000 L-fibreglass tank, where they were acclimatized for one week at ambient temperature (19-21°C). The fingerlings were fed with a locally formulated experimental diet containing 32% CP at 3% body weight. At the end of the acclimatization period, fish were starved for 24hrs and anaesthetized using FA 100 (4-allyl-2-methoxyphenol), reweighed and a total of 480 uniform size fish (5.12 ± 0.11, mean weight ± s.d.; 5.48 ± 0.14cm, mean standard length ± s.d.) were pooled in a second 2000 L-blue fiberglass tank. In this highly aerated tank, fish spent a night before they were stocked early the following morning. There were 18 different transclucent circular tanks (200 L each) which were stocked at a stocking rate of 20 fingerlings/tank, and initial average weights in each tank was checked and was not significantly different among tanks. The experiment was a 3 by 2 factorial in a completely randomized design having three temperature levels (20°C, 25°C and 30°C) and two feeding levels (3% and 6% body weight), implying a triplicate experimental layout. The experimental water temperatures were raised gradually by 2°C/day by using Tetratec® HT 200 automatic aquarium heaters, following a similar procedure by Kang'ombe (2004).

Rearing conditions

A static-water aerated system was used in this experiment whereby two-thirds of water was replaced every two days. In order to reduce incidences of stress-related mortalities, sampling and cleaning of tanks were synchronized every two weeks throughout the experiment. Fish were fed twice daily (at 09:00 and 18:00 hrs) with pellet diet at 3% or 6% bodyweight depending on treatment and feeding quantities were adjusted accordingly in relation to biweekly weights. The feed ingredients comprised of fishmeal, soybean meal, maize bran, vegetable oil, vitamin and mineral premixes, while snowflake wheat cake flour was used as binder (for complete feed formulation, see Ssebisubi, 2008, unpubl. thesis). During biweekly sampling, the weight and standard length of fish were measured. Standard length was measured to the nearest mm by using the stainless steel topped board whose plate ensured minimal drag and loss of scales during measurement, while weight was measured to 0.01g by using an electric analytical balance (Yoshida model AND). To minimize stress during biweekly measurements, the fish were anaesthetised by FA 100 bath (1 ml/5 litres) for 60 seconds. In all treatments, water temperature and pH were measured daily at 08:00

and 16:00 hrs, and ammonia levels were monitored weekly, while dissolved oxygen was measured twice per week. Throughout the experiment, which is 14 weeks, mercury thermometers were suspended in the experimental tanks to ensure stability of daily water temperature.

Data collection and statistical analysis

For geometric morphometrics data, only fish that survived by the termination of the experiment were used. Body shape was quantified using landmark-based geometric morphometric (GM) methods (Rohlf & Marcus, 1993). Image of each specimen was taken using a CANON digital camera, with a resolution of 7.1 megapixels, which was fixed at 30cm above the specimen on a tripod stand. To facilitate accurate placement of landmarks on all specimens, pins were placed on each specimen before image acquisition and data collection. The x, y coordinates of thirteen homologous landmarks (Fig. 1) were digitized from the left side of each individual using TPSDIG2 (Rohlf, 2004a). The biological names and descriptions of each landmark used can be found in the figure legend of Figure (1) while the sample size for each treatment combination can be seen in Table (1). To obtain shape variables, non-shape variation in the landmark coordinates was removed by superimposing them using a Generalized Procrustes Analysis (GPA) (Rohlf & Slice, 1990). GPA removes non-shape variation by scaling all specimens to unit size, translating them to a common location, and rotating them so that their corresponding landmarks line-up as closely as possible. The above procedures were implemented in TPSRELW (Rohlf, 2004b).

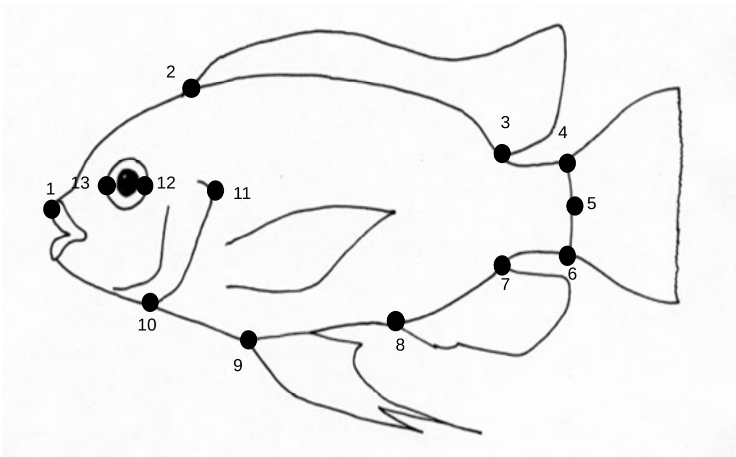

Fig. 1: Landmarks collected from the left side of each specimen, 1: tip of the premaxilla; 2, 3: anterior and posterior insertion of the dorsal fin; 4, 6: upper and lower insertion of caudal fin; 5: Posterior end of the lateral line; 7, 8: posterior and anterior insertion of the anal fin; 9: insertion of the pelvic fin; 10: insertion of the operculum on the body profile; 11: posterior extremity of the operculum; 12, 13: posterior and anterior end points of the eye diameter.

First, to determine if shape varied significantly among treatments, a two-factor multivariate analysis of variance (MANOVA) was performed, with temperature and feed rate as main factors and their interaction. In the case of significant differences being revealed by MANOVA, pairwise multiple comparisons were performed to determine exactly which treatments significantly differed from one another, after a Bonferroni correction. These were based on generalized Mahalanobis distance (D^2)

from a canonical variates analysis (CVA). Secondly, to determine if size differed significantly among treatments, a centroid size was subjected to a two-factor analysis of variance (ANOVA). MANOVA and CVA were computed with NTSYS-PC, version 2.1 (Rohlf, 2000) while ANOVA was performed in SPSS, version 15.

RESULTS

Two-factor MANOVA revealed significant body shape variation among temperatures used (Wilk's $\lambda = 0.0800$, $P < 0.0001$), but not in feed rate (Wilk's $\lambda = 1.0000$, $P = 0.4678$) or the interaction between temperature and feed rate (Wilk's $\lambda = 0.9800$, $P = 0.3654$). And pairwise comparisons revealed that it was only fish reared at 20°C that differed significantly to both those reared at 25°C ($D^2 = 4.6928$, $P < 0.0001$) and 30°C ($D^2 = 4.3542$, $P < 0.0001$) while those at 25°C and 30°C did not statistically significantly differ in body shape ($D^2 = 3.7717$, $P > 0.05$). To visualize body shape differences depicted by the above statistical results, thin-plate spline deformation grids were generated (Fig. 2), representing consensus configurations for each treatment (i.e. temperature and feedrate combinations). For those fish reared at 20°C, they tend to have shorter but deeper bodies, considering the inward displacement of landmarks 1 and 5 for body length and outward displacement of landmarks 2 and 9 for body depth, as opposed to the longer and slender bodies found in those reared at 25°C and 30°C. Another major difference also occurs when landmarks 12 and 13 are considered, whereby fish reared at 20°C have larger eye diameter than those at 25°C and 30°C. And finally, those reared at 20°C have longer heads, considering the posterior displacement of landmark 11, than those reared at 25°C and 30°C.

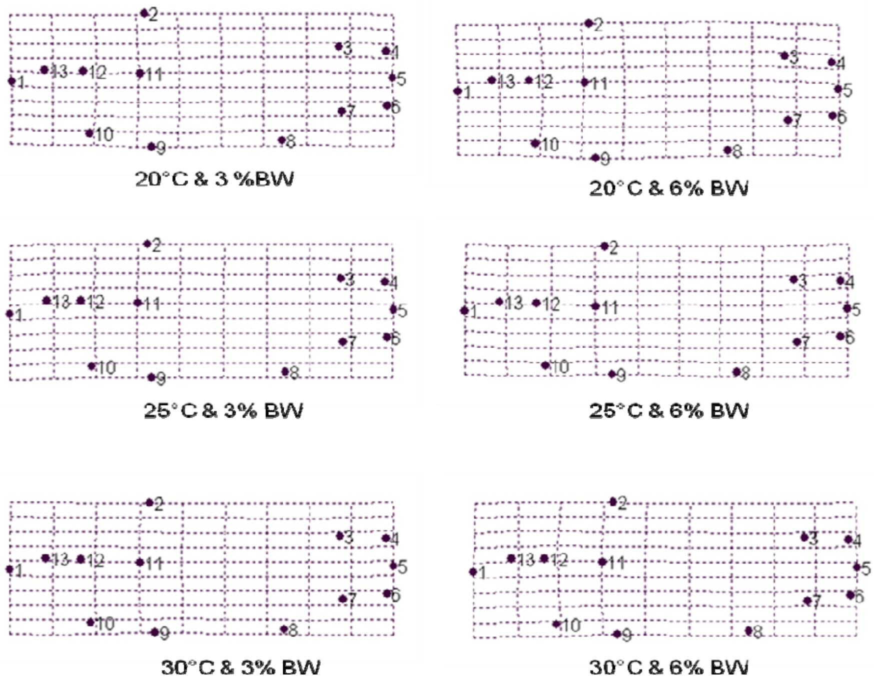

Fig. 2: Thin-plate spline deformation grids representing consensus configurations for each treatment combination used to describe shape variation among culturing environments. BW represents body weight.

Two-factor ANOVA revealed significant differences in body size among temperatures ($F = 117.74$, $df = 2$, $P < 0.0001$), feedrate ($F = 4.52$, $df = 1$, $P = 0.0345$) and also their interaction ($F = 10.43$, $df = 2$, $P < 0.0001$). In terms of temperature, the general trend is that those fish reared at 20°C are the smallest in terms of body size (mean ± SE = 673.4 ± 8.3) followed by those reared at 25°C (mean ± SE = 732.4 ± 8.2) and the largest being those at 30°C (mean ± SE = 848.5 ± 7.6). As for feedrate, those fish fed at 3% body weight had smaller body size (mean ± SE = 739.3 ± 6.9) than those fed at 6% body weight (mean ± SE = 773.3 ± 6.3). The posthoc pairwise comparisons reveals how temperature and feedrate interacted in affecting the body size of the fish (Table 1). From this table, it can be seen that fish raised at 20°C and 25°C, they tend to have similar body sizes regardless of the feedrate, thus feeding fish at 6% body weight did not have any advantage over the 3% body weight feedrate. Although the general trend has been that those fish fed at 3% body weight had smaller body sizes than their 6% body weight counterparts, it has been seen that those fish raised at 25°C showed a departure from this norm, in a way that those fish fed at 3% body weight had slightly larger body size (753.9±12.7) than those fed at 6% body weight (717.1±10.8). At 30°C, fish fed at 3% body weight were significantly smaller than those fed at 6% body weight.

Table 1: Pairwise comparisons on centroid size among treatments. Numbers in parentheses, in the columns, represent mean ± SE and in the rows, represent sample size, n.

Treatment	20&3 (658.3±11.4)	20 & 6 (689.8±11.9)	25 & 3 (753.9±12.7)	25 & 6 (717.1±10.8)	30 & 3 (810.4±11.6)	30 & 6 (875.4±9.8)
20 & 3 (37)	0					
20 & 6 (34)	-31.4	0				
25 & 3 (30)	-95.7**	-64.2**	0			
25 & 6 (42)	-58.7**	-27.3	36.9	0		
30 & 3 (36)	-152.2**	-120.7**	-56.5**	-93.4**	0	
30 & 6 (51)	-217.1**	-185.6**	-121.4**	-158.3**	-64.9**	0

** indicates highly significant difference among the means, P<0.001. On treatments, for example 20 & 3, denotes fish raised at 20°C and fed at 3% body weight.

And for water quality parameters measured, dissolved oxygen decreased with increasing temperatures, for instance the ranges at each temperature were as follows: at 20°C (7.45-7.53mg/l), at 25°C (6.89-7.07mg/l) whilst at 30°C it was 6.49-6.50mg/l. The pH range for whole experiment was 6.84-6.96, whilst total ammonia ranged from 0.11 to 0.63. All these parameters were within normal range for the culturing of this species.

DISCUSSION

Geometric morphometrics has revealed its robustness and usefulness as a tool for aquaculturists in assessing fish quality in terms of body shape and size in relation to environmental conditions under which the fish have been raised. This study, the first of its kind to be applied on Malawian cultured fish species, has revealed very important differences among fish raised at different water temperatures and fed at different rates. In general, the fish that were raised at 20°C, regardless of the feedrate, had shorter but deeper bodies, larger eyes and longer heads as opposed to the longer and slender bodies, smaller eyes and shorter heads found in those reared at 25°C and 30°C. In terms of size, fish reared at 20°C were the smallest, followed by those at 25°C and then the largest were those at 30°C.

Oreochromis shiranus has been reported to grow best when raised between 22°C and 31°C (Kiyoshi *et al.*, 2004), a finding which is concordant with the results found in this study since those fish reared at both 25°C and 30°C were larger and showed no significant difference between them in terms of shape. The fact that raising fish at either 25°C or 30°C does not affect body shape is a very important revelation considering the wide range of temperatures in areas where this fish is cultured throughout Malawi. Poikilotherms need heat for increased activity and metabolism as several authors have reported the positive effect of water temperature on growth as long as the increase is within the optimal range for biological activity (Gilbert, 1994; Jobling, 1995; Van Ham *et al*., 2003). Voluntary feeding by any individual fish is dictated by the appetite the fish has. The appetite peaks when water temperatures approach the upper thermal tolerance limit of the fish species and it falls dramatically above and below the optimum. This implies that the better growth performance evidenced for those fish raised at 25°C and 30°C can be explained by the fact that the fish had increased appetite and thus were able to utilize all the food provided. Growth is a result of tissue deposition and such deposition is high when food availability is optimal (Jobling, 1995). The results revealed in this study, i.e. feeding effects on growth of *O. shiranus* being enhanced with an increase in temperature, augers well with what Van Ham *et al*. (2003) reported, thus metabolism is enhanced by water temperature.

The smaller sizes of fish raised at 20°C has to do with the stress the fish is in due to such low temperature. Such finding is concordant with what Chaula *et al*. (2002) found, thus there is minimal feeding by *O. shiranus* when temperatures drop below 21°C. And our results indicate that no matter how much food you may provide to your fish, growth will not improve as long as temperatures are below 21°C. However the revelation that feeding fish at 6% body weight, raised at 25°C, could not improve growth, as indicated by smaller size of fish at 6% than those at 3%, is something very strange, and calls for further investigation since the expectation was to get larger fish for those fed at 6% body weight. This finding is similar to what Ssebisubi (2008, unpubl. thesis) found whereby those fish raised at this temperature did not show any significant difference in final body weight whether fed at 3 or 6% body weight. Those fish raised at 30°C but fed at 3% body weight were smaller than their counterparts at 6% body weight because metabolism at 30°C was higher but the 3% feedrate was not sufficient enough to meet the demands of the fish on food, unlike the 6% feedrate. This means that if a farmer has to realize higher returns in terms of size, the fish raised at 30°C must be fed at 6% body weight to meet the body demands of the fish for proper growth.

In conclusion, this study has revealed that raising fish at either 25°C or 30°C does not affect fish shape in any way, and that no fish was deformed, which is good for consumers since visual perception by consumers on shape influences the marketability of fish. And this also means that *O. shiranus* can be raised at a wide temperature range without compromising its shape. Of course raising fish at 30°C and feeding them at 6% body weight is the best combination to get higher returns from *O. shiranus* farming. And based on these results, aquaculturists can be encouraged to utilize this geometric morphometrics technique in assessing fish quality as well as the growth performance of fish, more especially these days with advanced technology which allows one to get digitized images for analysis without killing the fish itself.

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