SHORT COMMUNICATION

Fluctuating asymmetry in genetically improved Nile Tilapia, *Oreochromis niloticus* (Linnaeus), strains in the Philippines

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Fluctuating asymmetry (FA), a subtle random deviation between the left and right sides of symmetrically bilateral characters, is an easy and cost-effective tool for monitoring both genetic and environmental stressors that could undermine the overall health condition of an organism. In this study, we compared FA levels of thirteen bilateral characters in three common genetically improved Nile tilapia (GINT) strains in the Philippines, namely: GenoMar Supreme Tilapia (GenoMar), Genetically Improved Farmed Tilapia (GIFT), and Freshwater Aquaculture Center Selected Tilapia (FaST) strains. We found significantly higher FA levels in the GenoMar strain for opercular length and gill rakers. There was no significant difference in FA levels between the FaST and the GIFT strains for all the characters considered. Our findings indicate that the GenoMar strain may be experiencing higher stressors which are most likely due to the very high selection intensity done where only few and fast-growing individuals were included in the selection. Strong directional selection can result in loss of genetic variability, which is important in maintaining developmental stability. However, it is also possible that the stressors that might have caused higher FA levels in the GenoMar strain could be environmental in origin since the samples of the three GINT strains that we used in this study were reared in different environments. Thus, more studies are needed to elucidate further the relationships between FA levels and different stressors associated with the selection and rearing techniques used in GINT production. We also highly encourage further studies that will improve the effectiveness of FA as a tool for monitoring the levels of different stressors that may be undermining the long-term sustainability of GINT production in the country.

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INTRODUCTION

Research on the genetic improvement of the Nile tilapias (GINTs) in the last three decades dramatically boosted tilapia production in the Philippines (Dey 2000). GINTs have become popular among local tilapia farmers because of their superior performance traits (i.e. growth rate and size at maturation) compared to wild and unselected strains (Basiao and Doyle 1999; Bolivar and Newkirk 2002; Eknath et al. 2007; Romana-Eguia et al. 2010; Santos et al. 2014). With the increasing importance of GINTs in Philippine aquaculture, it is essential to monitor stressors affecting the fitness of the stocks that may be undermining long-term production sustainability. Selective breeding techniques that have been done for over a decade may have reduced the genetic variability of GINTs.

KEYWORDS
genetically improved Nile tilapia, aquaculture, fluctuating asymmetry, stress, developmental stability

Fluctuating asymmetry (FA), a subtle random deviation between the left and right sides of symmetrically bilateral characters, is the most commonly used measure of developmental stability (Clarke 1993; Rasmuson 2002). The degree of FA reflects the magnitude of stress, both genetic and environmental, that could disrupt the expected development of individuals. Studies have shown that an increase in FA levels is associated with an increase in stress levels (Palmer and Strobeck 1986; Clarke 1993; Rasmuson 2002). FA has also been found to be associated with some measures of fitness such as mortality and susceptibility to infections (Reimchen and Nosil 2001; Frechette et al. 2003; Moller 2006).

In this study, we compared FA levels based on multiple bilateral characters among three GINT strains developed and widely used in the Philippines. FA is an easy and cost-effective tool, which makes it a practical and useful stress-monitoring tool in aquaculture.

MATERIALS AND METHOD

Background on the development of the three GINT strains

The three GINT strains we used in our study were the GIFT or genetically improved farmed tilapia (Eknath et al. 1993), the GenoMar or GenoMar Supreme Tilapia (Gjoen 2001) and the FaST or Freshwater Aquaculture Center-selected tilapia (Bolivar and Newkirk 2002) strains. The GIFT strain was a synthetic strain developed initially in 1988 from a broad genetic base consisting of the best African (Egypt, Ghana, Kenya, Senegal) and Asian (Israel, Singapore, Taiwan and Thailand) Nile tilapia strains (Eknath et al. 1993). It was further developed through combined between-family and within-family selection (Gupta and Acosta 2004). The original stock of the GenoMar strain came from the Tilapia Breeding Nucleus of the GIFT Foundation when the GIFT Foundation went into a commercial alliance in 1999 with GenoMar, a private Norwegian company involved in aquaculture biotechnology (Ponzoni et al. 2010). GenoMar has improved the GIFT strain using DNA-assisted selection. Being a private sector, some details on the DNA-assisted selection techniques used in the development of GenoMar were not made known to the public (Ponzoni et al. 2010). The FaST strain on the other hand was developed through within-family rotational mating selection on pre-selected and size-graded stock (Bolivar and Newkirk 2002). This stock originated from a base population composed of the second generation of a fast-growing line of Nile tilapia whose founder stock consisted of Israel, Singapore, Taiwan and FAC strains (Abella et al. 1986).

Fish samples

A total of 170 Nile tilapia adults from three widely used GINT strains (GIFT, n = 53; GenoMar, n = 60; FaST, n = 57) in the Philippines were assessed for FA. The GenoMar and the FaST strains were procured from the breeding facilities at Central Luzon State University in Nueva Ecija, Philippines in March, 2007. The GIFT strain was procured from a grow-out farm in Pampanga, Philippines in August, 2007. All the fish samples were immediately brought to a laboratory where each fish was put inside a labeled cellophane bag and immediately stored and frozen overnight before being examined for FA.

Samples of the GenoMar strain were generally larger and older (SL, mean±sd: 161±18 mm; 7-8 mo old) than the samples of the GIFT strain (SL, mean±sd: 148±10 mm; 4 mo old) and FaST (SL, mean ±sd: 108±5 mm; 4-5 mo old) samples. Ideally, FA studies are made among populations with similar ages and body sizes although proper corrections for size can be done (Palmer 1994). There are a number of FA studies on populations with different ages and body sizes (i.e. Vollestad and Hindar 2001).

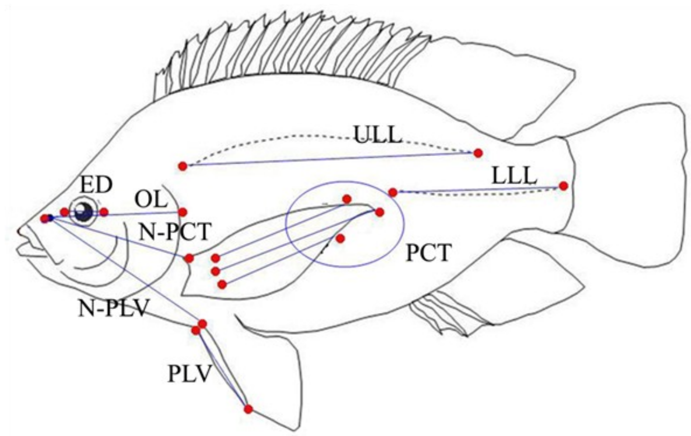

Figure 1. Schematic diagram of the morphometric characters. **PLV,** Pelvic spine. **PCT,** Pectoral fin (average length of 3rd, 4th and 5th pectoral fin rays). **ED,** Eye diameter. **OL,** Opercular length. **N-PCT,** Length from nostril to insertion of pectoral fin. **N-PLV,** Length from nostril to insertion of pelvic spine. **LLL,** Lower lateral line. **ULL,** Upper lateral line.

Eight morphometric (Figure 1) and five meristic bilateral characters were assessed for FA. We used multiple characters because previous studies have shown that FA-stress association is more likely to be detected when FA is assessed based on multiple characters since different characters tend to have varying degrees of developmental stability (Palmer 1994; Leung et al. 2000).

The meristic characters were: 1) number of pelvic fin rays (**PLVFR**), 2) number of pectoral fin rays (**PCTFR**), 3) number of gill rakers of the first upper gill arch (**UGR**) and 4) number of gill rakers of the first lower gill arch (**LGR**), and 5) total number of gill rakers (**GR**) which is the sum of the upper and lower gill rakers counts. For **PLVFR** and **PCTFR**, the number of rays was counted at the bases of fins. All characters were chosen because either they have been used in previous studies or are easy to measure.

Each fish was thawed in tap water before morphometric measurements were taken. All measurements on the left and right sides of bilateral characters were taken with a digital caliper to the nearest 0.01 mm. To minimize observer-generated bias in measurement, all measurements of the left side were taken after measurements of the right side were completed so that right and left measurements of each bilateral character for each fish were not taken successively. Each fish was frozen after measurements were taken. Each morphometric measurement was taken twice at four-day intervals to minimize measurement error. After all the measurements for morphometric characters were completed, the pelvic fins, pectoral fins and gills were dissected from the left and right side of each fish and placed in labeled bottles containing 5% formalin solution. Meristic characters were counted twice at daily intervals under a dissecting microscope. All the measurements for both morphometric and meristic characters (Table 1) were done by one person to ensure consistency. Damaged or deformed characters were excluded from the analysis.

Table 1. Size of characters and number of tilapias measured for each character among GenoMar, GIFT and FaST (N (mean ± standard deviation). Values are expressed in mm and counts for morphometric and meristic characters, respectively.

Character	GenoMar	GIFT	FaST
Morphometric			
PLV	60 (26.39±2.40)	53(23.88±1.59)	57(16.84±1.17)
PCT	60 (52.31±6.03)	53 (46.23±2.80)	57 (34.33±1.87)
ED	60 (15.19±1.12)	53 (12.36±0.48)	57 (12.14±0.61)
OL	60 (48.93±4.77)	53 (41.35±2.36)	57 (33.38±1.46)
N-PCT	60 (49.16±4.81)	53 (42.51±2.45)	57 (34.14±1.58)
N-PLV	60 (62.77±6.11)	53 (55.79±3.37)	57 (42.71±2.20)
LLL	58 (55.94±10.27)	53 (48.40±6.58)	57 (36.31±3.69)
ULL	59 (82.83±10.29)	53 (73.47±6.20)	56 (55.39±3.21)
Meristic			
UGR	48 (7.27±0.91)	42 (6.46±0.83)	46 (6.19±0.84)
LGR	48 (24.88±1.27)	42 (24.91±1.07)	46 (22.74±1.17)
GR	48 (32.16±1.66)	42 (31.36±1.36)	46 (28.93±1.45)
PCTFR	59 (14.26±0.43)	52 (14.00±0.24)	56 (13.90±0.45)
PLVFR	60 (4.99±0.06)	53 (4.99±0.07)	56 (4.98±0.13)

Statistical Analyses

FA1, the absolute value of the right measurement minus the left measurement ($\left| R - L \right|$), was used for FA level comparison among the strains. When a significant positive correlation between FA and character size existed, a size-scaled FA index (FA2) was used. FA2 is the proportion of FA1 of a fish of a certain strain for a given character to the mean character size of all the fish in that strain ($\left| R - L \right| / \text{Mean } (R + L) / 2$). Regression and Pearson correlation analyses of FA1 and mean character size revealed significant positive correlation between mean character size and three characters, namely: eye diameter (R = 0.23, P <0.05), opercular length (R = 0.408, P < 0.01) and lower lateral line (R = 0.411, P < 0.05). Thus, FA2 was the index used for FA comparison among strains in these three characters while FA1 was the index used for the rest of the characters.

Characters that exhibited directional asymmetry and antisymmetry and where FA level was not significantly higher than measurement error were excluded in the analysis. One-sample t-test of right minus left (R-L) values against a mean of zero was used to test for the presence of directional asymmetry. Kurtosis level and visual inspection of R-L distribution were used to determine the presence of AS.

The difference between the left and right sides of a bilateral character are sometimes too small (Palmer and Strobeck 1986). Thus, to ensure that the observed differences between the right and left characters were FA, and not simply the result of measurement errors, a mixed model ANOVA was done where we treated measurements as dependent variables, individual (fish) as random factor and sides as fixed effects.

There was no significant difference in FA levels between male and female individuals for all characters. Thus, we pooled all the individuals by strain in the analyses. ANOVA was used to compare FA level among strains for each character. Results from ANOVA were subjected to sequential Bonferroni corrections. For among strains FA level comparison, *Post hoc* Tukey's test was done for characters that yielded significant results.

FA level was also compared among the strains across all characters that exhibited FA distribution following composite fluctuating asymmetry (CFA) analysis outlined by Leung et al. (2000). Only fish with complete FA measurements for all characters were included in CFA analyses. Since different characters tend to have different FA magnitudes, standardization of the data was done by dividing each FA value for a given character by the average FA values of a given character across all the fish in the three strains (FA_i / Mean FA_{ij}). This ensured that all characters considered contribute equally to CFA measures. The new FA values were then summed across all characters for each fish so that each fish had a composite FA score. Since **GR** was the pooled counts of **UGR** and **LGR**, two CFA scores were computed. One CFA score (CFAa) was the summed FA values of **GR** and all other characters that exhibited FA excluding **UGR** and **LGR**. The other CFA score (CFAb) was the summed FA values of **UGR**, **LGR** and all other characters excluding **UGR**. One-way ANOVA was used to compare CFA levels among strains.

All analyses were done according to Palmer (1994) except for the composite fluctuating asymmetry index, which was done according to Leung et al. (2000). All statistical analyses were implemented using the SYSTAT 6 (Wilkinson 1996) statistical software.

RESULTS AND DISCUSSION

A mixed-model ANOVA showed that FA level was significantly larger than measurement error for all characters (P < 0.05) except for N-PLV in the GIFT strain. Right minus left (R-L) values for most of the characters exhibited FA distribution (Tables 2a and 2b). N-PCT in the GIFT and the FaST strains exhibited directional asymmetry as indicated by R – L mean that is significantly different from zero. N-PLV and N-PCT were therefore excluded from further analyses. Test for kurtosis showed that most of the R-L distributions were leptokurtic or

Table 2a. Summary of asymmetry characteristics of the morphometric characters for the three Genetically Improved Nile Tilapia strains.

Character		GenoMar	GIFT	FaST
PLV	N	60	53	57
	Mean±SD	-0.089 ±0.944	-0.148±0.796	0.048±0.940
	t-test	NS	NS	NS
	Kurtosis	1.497	10.805	39.045
	Type	FA	FA	FA
PCT	N	60	53	57
	Mean±SD	0.255±1.566	-0.014±1.261	-0.031±1.277
	t-test	NS	NS	NS
	Kurtosis	9.008	1.003	5.719
	Type	FA	FA	FA
ED	N	60	53	57
	Mean±SD	-0.106±0.552	-0.044±0.287	0.013±0.315
	t-test	NS	NS	NS
	Kurtosis	4.955	0.173	-0.228
	Type	FA	FA	FA
OL	N	60	53	57
	Mean±SD	-0.140±0.849	0.097±0.371	-0.012±0.342
	t-test	NS	NS	NS
	Kurtosis	0.524	2.177	-0.479
	Type	FA	FA	FA
N-PCT	N	60	53	57
	Mean±SD	-0.183±1.204	-0.540±0.958	0.374±0.775
	t-test	NS	**	**
	Kurtosis	1.522	1.518	0.171
	Type	FA	DA	DA
LLL	N	58	53	57
	Mean±SD	-0.085±7.459	-1.220±6.468	-0.927±3.786
	t-test	NS	NS	NS
	Kurtosis	2.045	5.753	0.598
	Type	FA	FA	FA
ULL	N	59	53	56
	Mean±SD	1.543±9.198	-0.055±3.462	0.460±3.051
	t-test	NS	NS	NS
	Kurtosis	26.716	-0.072	2.531
	Type	FA	FA	FA

t –test, H_0: mean = 0.0
* $P < 0.05$; ** $P < 0.01$; NS = not significant

Table 2b. Summary of asymmetry characteristics of the meristic characters for the three Genetically Improved Nile Tilapia strains.

Character		GenoMar	GIFT	FaST
UGR	N	48	42	42
	Mean±SD	0.042±0.798	-0.167±1.010	0.033±0.710
	t-test	NS	NS	NS
	Kurtosis	1.451	0.820	-0.688
	Type	FA	FA	FA
LGR	N	48	42	42
	Mean±SD	-0.021±1.271	0.262±1.308	-0.087±1.002
	t-test	NS	NS	NS
	Kurtosis	0.675	-0.523	1.925
	Type	FA	FA	FA
GR	N	48	42	46
	Mean±SD	0.021±1.611	0.226±1.236	-0.054±0.973
	t-test	NS	NS	NS
	Kurtosis	-0.561	1.294	1.309
	Type	FA	FA	FA
PCTFR	N	59	52	56
	Mean±SD	0.085±0.501	0.038±0.394	-0.080±0.520
	t-test	NS	NS	NS
	Kurtosis	1.059	3.910	3.512
	Type	FA	FA	FA
PLVFR	N	60	53	56
	Mean±SD	0.017±0.129	-0.019±0.137	0.036±0.267
	t-test	NS	NS	NS
	Kurtosis	60.000	53.000	56.000
	Type	FA	FA	FA

t –test, H_0: mean = 0.0
* $P < 0.05$; ** $P < 0.01$; NS = not significant

Table 3. ANOVA to compare FA of GenoMar, GIFT and FAST strains for each character and across all characters (CFA) that exhibited FA distribution.

Character (FA index)	N	Df	MS	F	P	Rank
PLV (FA1)	170	2	1.096	2.157	NS	
PCT (FA1)	170	2	0.364	0.344	NS	
ED (FA2)	170	2	9.305	2.390	NS	
OL (FA2)	170	2	7.099	10.719	**	GenoMar[a]>FaST[b]>GIFT[b]
LLL (FA2)	168	2	34.411	0.490	NS	
ULL (FA1)	168	2	111.245	4.478	NS*	GenoMar[a]>GIFT[a, b]>FaST[b]
UGR (FA1)	136	2	0.383	0.940	NS	
LGR (FA1)	136	2	1.393	2.355	NS	
GR (FA1)	136	2	5.000	6.715	*	GenoMar[a]>GIFT[a, b]>FaST[b]
PCTFR (FA1)	167	2	0.161	0.880	NS	
PLVFR (FA1)	169	2	0.006	0.173	NS	
CFAa	129	2	134.026	1.824	NS	GenoMar>FaST>GIFT
CFAb	129	2	71.507	0.967	NS	GenoMar>FaST>GIFT

* $P < 0.05$; ** $P < 0.01$; NS = not significant; NS* Significant ($P < 0.05$) before adjusted by Bonferroni correction; [a,b,c] same letters indicate no significant difference; different letters indicate significant difference **CFAa**, summed FA values of GR and all other characters except UGR and LGR. **CFAb**, summed FA values of UGR, LGR and all other characters except GR.

close to zero. Some characters that exhibited platykurtosis had a low level kurtosis (≥ -0.688). Generally, test for kurtosis in addition to visual inspection of R-L distributions indicated that no character exhibited antisymmetry.

Only **OL**, **ULL** and **GR** showed significant differences in FA level among strains (Table 3). Nonetheless, after sequential Bonferroni corrections, only **OL** and **GR** remained significantly different. *Post hoc* Tukey's test revealed that FA level for **OL** in the GenoMar strain was significantly higher than in both the GIFT and FaST strains. For **GR**, FA in the GenoMar strain was significantly higher than that in the FaST strain. The GIFT strain was not significantly different from either the GenoMar or FaST strain for **GR**. No significant difference was detected in FA levels between the GIFT and FaST strains for all the characters. Composite fluctuating asymmetry analysis (CFA) did not reveal any significant difference in FA levels among the three strains although mean values showed high CFA level in the GenoMar strain than in the other two strains (Table 3).

Fluctuating asymmetry is hypothesized to arise from both genetic and environmental stressors during the development of an individual and is related to a reduced overall health condition. Thus, the differences in FA levels among the three GINT strains may indicate that FA is sensitive enough to detect the differences in the levels of stressors that might have differentially affect the normal development of bilateral characters in the three GINT strains. The results of our study further indicate that, except for N-PCT and N-PLV, all the characters we considered in this study can be useful for future FA studies involving cultured tilapia in the Philippines.

The significantly higher FA levels in the GenoMar strain, at least for OL and GR characters, than the other two strains suggest that GenoMar samples may have experienced higher level of stressors during their development. Based on some available information from the literature, we can only hypothesize that the more intense selection techniques used in the development of the GenoMar strain might explain the high FA levels observed. Since the founder population (i.e. original breeding stock) of the GenoMar strain was composed of only few fast growing GIFT individuals, strong directional selection for some genes may have happened in the development of GenoMar strain resulting in the loss of genetic variability important in maintaining developmental stability (see Crozier et al. 1997; Calcagnotto and Toledo-Filho 2002; Hoelzel et al. 2002). High selection intensities applied to achieve high genetic gains implies that the number of individuals that are mated to contribute to successive generations becomes fewer and the probability of mating related individuals increases particularly in mixed-size hatchery stocks. However, it is also possible that the stressors that might have caused higher FA levels in the GenoMar strains could be environmental in origin since the samples of the three GINT strains that we used in this study were reared in different hatcheries/fish cages (see Fessehaye et al. 2007). Further studies are needed to investigate the specific factors that could explain the differences in FA levels among the three GINT strains.

Future studies should investigate the association between FA levels and the levels of stressors, both genetic and environmental, that will have negative impacts on the fitness or overall health condition of the GINTs such as survival rate, susceptibility to diseases and other health anomalies associated with inbreeding depression that usually arise from very intense selection techniques. It is also important to investigate how specifically FA levels in different symmetrical characters are associated with different types of stressors. For example, our study revealed varying FA levels among the thirteen studied bilateral characters, which indicate that different characters have varying levels of developmental stability (Palmer 1994; Leung et al. 2000). It is also suggested that different characters may have different responses to different types of stressors (Crozier et al. 1997; Leamy and Klingenberg 2005). In this way, we can easily identify the stressors and make some necessary improvements in the selection or rearing techniques in order to maintain healthy stocks for sustained GINT production the country. We further recommend that future studies should investigate FA levels of different characters individually rather than collectively using composite FA index. Using composite FA index may reduce the sensitivity of FA as a tool as more developmentally stable characters tend to mask the FA levels exhibited by some characters (Palmer 1994; Leung et al. 2000).

With the declining fish production from capture fisheries (Costello et al. 2012; Muallil et al. 2014a, 2014b), the aquaculture sector has become an important source of fish protein. The world tilapia production has considerably increased from about 830,000 mt in 1990 to about 3.5 million mt in 2008 (Josupeit 2010). The Philippines, as the fourth top tilapia producing country in the world, has produced about 260,535 mt of tilapia in 2012 (BFAR 2014). The development of GINTs has contributed a lot to the increase in tilapia production. However, as we pursue to improve further some of the more economically important traits such as fast growth, perhaps it is also important to carefully investigate the unwanted effects of selection techniques on survival rates and overall fitness of the fish (i.e. Fessehaye et al. 2009; Skaarud et al. 2011; Naish et al. 2013). Thus, we highly encourage further studies that will improve the effectiveness of FA, which is cost-effective and easy to implement, as a tool for monitoring the levels of different stressors that may be undermining the long-term sustainability of GINT production in the country.

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CONFLICTS OF INTEREST

There are no conflicts of interest.

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