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# Fluctuating Asymmetry: A Biomarker for Environmental Stress in *Gambusia affinis*

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**Abstract:** *Fluctuating asymmetry (FA), random and subtle differences occurring between the left and right sides of a normally bilaterally symmetrical organism, has been of interest to biologist who study the effects of and responses to environmental stress on organisms. The study aimed to assess the occurrence of FA in two meristic and six morphometric traits of the Gambusia affinis (Baird and Girard 1853). Gambusia affinis, the Western Mosquitofish, was collected from two waterways in Texas. Fish from Clear Creek, a spring-fed site with high dissolved oxygen, low turbidity, constant temperature and pH levels were compared to those from Diamond Y, a spring subject to high dissolved oxygen, high turbidity, high temperature fluctuations and pH levels. All fish (n=25) were assessed for the eight FA indices. Individuals collected from Diamond Y showed considerable difference in FA as compared to the Clear Creek fish. Overall, five of the eight FA traits showed significant differences in assessing deviations from symmetry. These findings demonstrate FA as a useful tool for detecting biological changes in stressful environments.*

***Keywords:*** *Fluctuating asymmetry, Gambusia affinis, environmental stress, bioindicator, mosquitofish*

## 1. INTRODUCTION

Developmental homeostasis is the ability of organisms to maintain their course of behavior over age or time in the presence of environmental variability. This type of developmental stability is influenced by an organism's ability to handle environmental and genetic disturbances during development. Environmental stress has been shown to increase developmental instability. Studies that evaluate developmental instability allow conservation biologist to visualize a population's ability to survive change within their environment despite their genetic makeup [1]. Increase of developmental instability caused by genetic of environmental stress can most often be displayed by evaluating fluctuation asymmetry (FA) – random and subtle deviations from bilaterally symmetrical traits [2]. Though some studies assess the influence of environmental and genetic stress on development by evaluating fertility and mortality, they require marked modifications over a longer period of time. Therefore, before a population has been obviously affected by such influence, fluctuating asymmetry has been proposed as an early warning biomarker in identifying potential harm of exposure to stress [3,4,5]. FA studies are being increasingly used over traditional methods for environmental and biomonitoring assessments [6,7, 8].

The study measured fluctuating asymmetry in two populations of western mosquitofish (*Gambusia affinis*). The western mosquitofish has been attractive to many researchers due to its ability to utilize a wide range of habitats and its occurrence across a broad thermal spectrum, from ice-covered lakes and ponds [9, 10] to thermally elevated springs and streams [11, 12]. Thus, the western mosquitofish is a good species to test whether FA can serve as a biomarker – a functional measure of exposure to various stressors , acting as an early warning sytem to assess declines in environmental quality and population health [13].

Waterways where the fish inhabit are both springs located in Texas. Clear Creek arise from the Edwards-Trinity aquifer [14]. Average water temperatures were 20.5 °C, average dissoled oxygen above 5 mg/L, pH near 7 and relatively low turbidity [15]. Due to its constancy in these paramenters, for this study the spring is categorized as an unstressed environment. Diamond Y is another spring located in Pecos County. The spings are thought to originate from the Permian Rustler Formation and issue from Cretaceous caronate rocks [16]. Spring parameters included average temperatures of 17.6 °C, dissolved oxygen up to 4 mg/L, pH at 7.6 (fluctuating), high ammonia levels and increased salinity. The site was categorized as a stressful environment. The objective of the study was to test the hypothesis that stress associated with development in an enviroment prone to perturbations

 

would increase developmental instability, which would be reflected in an elevated level of fluctuating assymetry in mosquitofish in Diamond Y as compared to Clear Creek.

## 2. MATERIAL AND METHODS

### 2.1 Sample sites

Western moquitofish were collected from Clear Creek, 16 km west of Menard, Menard County Texas and from Diamond-Y, 10 km north of Fort Stockton, Pecos County.

### 2.2 Fish

The study used mature female moquitofish from each spring locality.. Fish utilized in the present study were initially preserved in 95% ethanol for use in a previous study by Hubbs [15]. A random sample of 25 mature female fish was taken from each sample collection and used in the study.

### 2.3 Traits

Digital calipers (Mitytoyo series 500, Aurora, IL) were used to measure eight meristic and 3 morphometric traits (Table 1). Each fish was measured by a single individual and each measurement done twice on separate days to minimize error [17, 18]. The two meristic traits assessed were number of scales in the lateral line and number of pectoral fin rays while the six morphometric traits evaluated were length of fifth pectoral fin ray, orbit diameter, distance from eye socket to the base of the pectoral fin, body depth, standard length and total length.

**Table 1:** *Morphological traits used to measure FA*

| Trait | Abbreviation | Description |
|---|---|---|
| Numbers of scales in the lateral line | LLScales | All scales in the lateral line were counted |
| Number of pectoral fin rays | RPec rays | All pectoral fin rays on the right were counted |
| | LPec rays | All pectoral fin rays on the left were counted |
| Length of fifth pectoral fin ray | RPR len | Distance from the tip of the ray to th base of the right fin |
| | LPR len | Distance from the tip of the ray to th base of the left fin |
| Orbit diameter | OR diam | Distance from anterior edge of the eye to the posterior edge of the eye socket |
| Distance from eye socket to the base of the pectoral fin | RE to P | Length of a straight line from the dorsal base of the pectoral fin to th edge of the right eye socket |
| | LE to P | Length of a straight line from the dorsal base of the pectoral fin to th edge of the left eye socket |
| Body Depth | BD | Vertical distance from the dorsal margin of the body to the ventral margin of the body |
| Standard Length | SL | Straight-line measurement taken from tip of the snout to the last vertebra supporting the tail fin |
| Total Length | TL | Straight-line measurement taken from tip of the snout to the end of the caudal peduncle |

### 2.4 Statistical Methods

This study employed a quantitative approach in addressing the research objective. Descriptive analyses were conducted on each of the two meristic and six morphometric variables associated with fish from each population. In order to control for size effects and potential directional asymmetry, individual FA was quantified as the difference between left (L) and right (R) measures divided by mean trait size [19]. Independent samples analysis was then conducted to compare Clear Creek (unstressed) and Diamond-Y (stressed) populations to determine if there were any statistically significant effect in FA between the two.

## 3. RESULTS

Table 2 shows that *Gambusia affinis* females exposed to stressed conditions were significantly different from unstressed female fish in five morphometric traits ($p$<0.03). None of the two meristic traits were statistically significant (p>0.99). The magnitude of difference between groups were high (d>0.8) as measured by Cohen's *d.*

**Table 2:** *Comparison of meristic and morphometric traits in unstressed and stressed fish (n=25)*

| Variable | *M* | *SD* | *t* | *df* | *p* |
|---|---|---|---|---|---|
| BD | | | | | |
| Stressed | 7.67 | 1.49 | 6.09 | 48.00 | 0.000* |
| Unstressed | 5.6 | 0.81 | | | |
| LE to P | | | | | |
| Stressed | 4.81 | 0.60 | 8.76 | 48.00 | 0.299 |
| Unstressed | 3.49 | 0.47 | | | |
| RE to P | | | | | |
| Stressed | 4.86 | 0.65 | 8.21 | 48.00 | 0.033* |
| Unstressed | 3.56 | 0.45 | | | |
| RPR len | | | | | |
| Stressed | 5.17 | 1.10 | 4.81 | 48.00 | 0.138 |
| Unstressed | 3.77 | 0.95 | | | |
| LPR len | | | | | |
| Stressed | 5.31 | 0.92 | 17.89 | 48.00 | 0.000* |
| Unstressed | 1.90 | 0.26 | | | |
| LLScales | | | | | |
| Stressed | 29.06 | 1.82 | 1.36 | 48.00 | 0.795 |
| Unstressed | 28.36 | 1.82 | | | |

| | | | | | |
|---|---|---|---|---|---|
| LPec rays | | | | | |
| Stressed | 12.70 | 1.31 | 25.29 | 48.00 | 0.964 |
| Unstressed | 3.34 | 1.31 | | | |
| RPec rays | | | | | |
| Stressed | 12.42 | 1.11 | 1.52 | 48.00 | 0.545 |
| Unstressed | 11.58 | 2.54 | | | |
| OR diam | | | | | |
| Stressed | 2.10 | 0.19 | 5.31 | 48.00 | 0.999 |
| Unstressed | 1.91 | 0.26 | | | |
| SL | | | | | |
| Stressed | 31.83 | 4.20 | 7.33 | 48.00 | 0.000* |
| Unstressed | 24.51 | 2.70 | | | |
| TL | | | | | |
| Stressed | 37.55 | 4.79 | 7.12 | 48.00 | 0.002* |
| Unstressed | 29.29 | 3.27 | | | |

## 4. DISCUSSION AND CONCLUSION

The study utilized an analytical approach in assessing the reliability of FA as a biomarker for environmentally induced developmental instability. The occurrence of increased developmental instability in a population experiencing temperature fluxes and high pH is not itself evidence of cause and effect. However, the results of the present study are consistent with the hypothesis that stress from exposure to stochastic environmental conditions increased the level of developmental instability in female mosquitofish from the stressed spring. Of the two meristic and six morphometric traits measured, a significant majority (n=5) of the morphometric traits showed higher fluctuating asymmetry in Diamond-Y (stressed) fish compared to those from the Clear Creek (unstressed). Numerous studies have suggested that developmental stability is decreased by low genetic variation, which may induce an increase in the degree of fluctuating asymmetry observed [20, 21]. It can be concluded that FA in mosquitofish can serve as a reliable and sensitive biomarker for environmental stress. Future studies will assess the genetic variation within each population (Clear Creek and Diamond-Y) and determine if there is a correlation with FA.

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