Fluctuating Asymmetry and Condition in Fishes Exposed to Varying Levels of Environmental Stressors

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ABSTRACT

The ability of an organism to combat developmental stress is known as developmental stability which can be assessed by measuring fluctuating asymmetry. Fluctuating asymmetry (FA) is the variation in bilaterally symmetrical traits. Deviations in bilateral traits from perfect symmetry may point to developmental stress. Environmental factors or stressors such as chemical pollution have been shown to decrease developmental stability and increase levels of FA in several studies, but links between FA and condition as a measure of fitness in the literature are rare and needed.

The purpose of this study is to determine if fluctuating asymmetry (FA) and correlates of fitness are good indicators of stress. Sites were selected at approximately 3, 6, 12, and 26 kilometer increments upstream and downstream from the Leaf River Pulp Mill, New Augusta, MS. Collections of approximately twenty fish from each of three species: the longear sunfish (*Lepomis megalotis*), the blacktail shiner (*Cyprinella venusta*), and the highfin carpsucker (*Carpiodes velifer*), were made in early summer, late summer, and late fall of 2006. Several morphometric measurements were taken from each fish to determine the degree of FA. These included lateral line scale count, pectoral and pelvic fin ray count, length of longest pectoral fin ray, eye diameter, and head length. Gonadosomatic index (GSI), percent lipids, fecundity, and length/weight residual were measured as correlates of fitness.

The purpose of this study is to address the following questions: 1) Is there any difference in measures of FA and condition that correlate with position (upstream or downstream) from the source? 2) Is there any difference in measures of FA and condition that correlate with water column position of fishes?

Keywords: toxic substances, water quality, nonpoint source pollution, fluctuating asymmetry

Introduction

When wood is processed to make pulp, large quantities of organic material are produced and often discharged into local streams by pulp mills. There is a high probability that effluent from pulp mills will contain chemical pollutants that will be released into the water along with particulate matter left over from the manufacturing processes (Pearson, 1972). Dioxin is one of the most toxic of all substances that have been tested. Dioxin is also significant byproduct of pulp manufacturing that has been found to cause deformities and cancer in many laboratory animals (Harrison and Hoberg, 1991; Zala and Penn, 2004).

A stressor is considered to be anything that disturbs an organism's homeostasis (Bonga, 1997). Stress that occurs during development (developmental stress) can have adverse effects on an organism's growth (Bonga, 1997; Campbell, 2003). The ability of an organism to combat developmental stress is known as developmental stability (Moller, 1997). One way to gauge developmental stability is by measuring fluctuating asymmetry (Van Valen, 1962; Felley, 1981;

Fluctuating Asymmetry and Condition in Fishes Exposed to Varying Levels of Environmental Stressors

Zuber and Schaefer

Kozlov et al., 1996; Simmons et al., 1999; Floate and Fox, 2000; Klingenberg et al., 2002;). Fluctuating asymmetry (FA) is variation in bilaterally symmetrical traits from perfect symmetry (Felley, 1980; Kozlov et al., 1996; Floate and Fox, 2000; Hogg et al., 2001). Bilateral traits are produced by the same genome. Hence, if an organism was not under stress during its development, one would expect perfectly symmetrical bilateral traits and no measurable FA (Campbell, 2003). Deviations from perfect symmetry could point to developmental stress (Hogg et al., 2001). Environmental factors or stressors such as chemical pollution and heavy metals have been shown to decrease developmental stability and increase levels of FA (Kozlov et al., 1996; Floate and Fox, 2000; Hasson and Rossler, 2002).

Fluctuating asymmetry may serve as a useful indicator of biological perturbations. Currently, one of the main methods used to determine communities or ecosystems under stress is by documenting the loss of indicator species (Chase et al., 2000). A problem with this practice is that organisms possess different characteristics that allow them to utilize resources differently, and the disappearance of certain indicator species may be a result of predation or population density pressures and not necessarily because of pollution or other environmental factors (Landres et al., 1988). FA may be a better technique for identifying populations or individuals under stress than other techniques such as presence or absence of indicator species.

The purpose of this study is to determine if FA and correlates of fitness change with distance from a source of contaminants. Body condition as a measure of fitness is expected to decrease with increasing stress levels, and levels of FA are expected to increase with increasing stress levels. Also, links between FA and condition as a measure of fitness in the literature are rare and needed. I propose to measure fluctuating asymmetries and putative correlates of fitness such as gonadosomatic index (GSI), fecundity, length/weight residual and percent lipids in three species of fishes, *Lepomis megalotis*, *Cyprinella venusta*, and *Carpiodes velifer*.

Study Species

Three species of fishes were chosen because they were found in high abundances in collections taken from the Leaf River by Ross (1990) and the National Council for Air and Stream Improvement (NCASI, 2002-2003): *Cyprinella venusta, Lepomis megalotis,* and *Carpiodes velifer*. More specifically these species were chosen because a representative from each trophic position was desired to determine if proximity to contaminants in sediments affects correlates of fitness and/or FA. *Cyprinella venusta*, the blacktail shiner, is found mostly in the water column, typically spawns during late spring and early summer, and feeds on mostly insect larvae (Hambrick and Hibbs, 1977). *Lepomis megalotis*, the longear sunfish, can be found in all levels of the water column, typically spawns during late spring and early summer (Berra and Gunning, 1972), and feeds on aquatic insects and other fishes (Applegate et al., 1967). *Carpiodes velifer*, the highfin carpsucker, is a benthic fish that spawns from early summer to early fall and feeds mostly on detritus and algae (Beecher, 1979). According to Funk (1955) and Baker and Ross (1981), *L. megalotis* has a limited home range. Berra and Gunning (1972) estimate the home range of *L. megalotis* to be roughly 21-61 m while the home ranges of *C. venusta* and *C. velifer* are both considered to be much greater.

Study Area

The Leaf River originates in Scott County, Mississippi and flows southward through Hattiesburg (Figure 1). It continues a southeastwardly course through George County where it joins the Chickasawhay River and forms the Pascagoula River. Sites were selected at approximately 3, 6, 12, and 26 kilometer increments upstream and downstream from the Leaf River Pulp Mill, New Augusta, MS (Figure 1).

Materials and Methods

Sampling was done in July, September, and December of 2006 around sand bars of the Leaf River in the study area using cast nets and seines. Seines were of variable sizes but

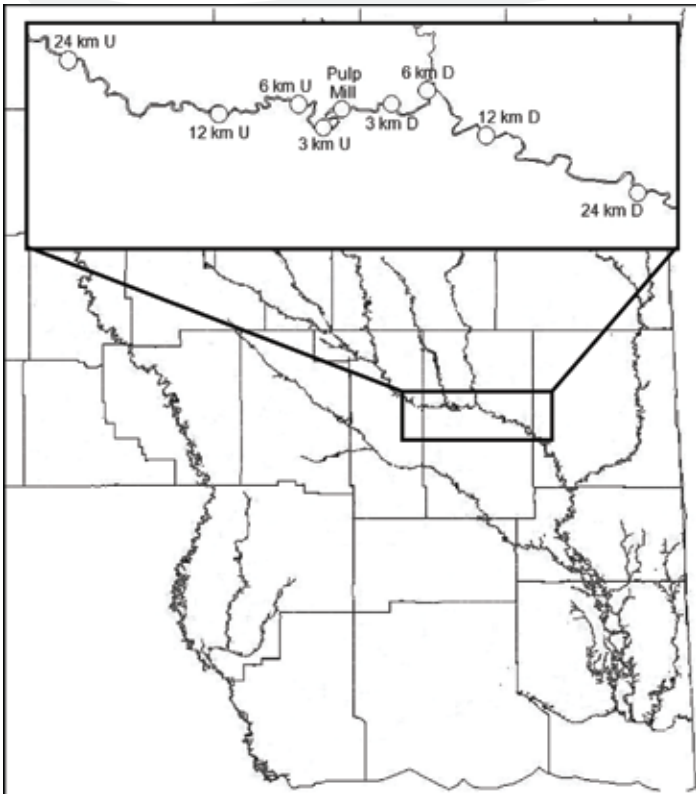

Figure 1. Map of southern Mississippi with study area on the Leaf River outlined in black. Inset shows the location of the sites in the study area. The letter U on site labels represents upstream sites, and the letter D represents downstream sites.

mesh was always 4.8 mm. Twenty individuals of each species were collected from each of the eight sites during each sampling trip. Fishes were fixed in 10% formalin immediately after collection and later transferred to 5% buffered formalin (Huelett et al., 1995).

Phenotypic Measurements and Measures of Condition

Several bilateral phenotypic measurements were taken from each fish, including lateral line scale count (Felley, 1980), pectoral and pelvic fin ray count (Gross et al., 2004), length of longest pectoral fin ray, eye diameter, and head length (Bryden and Heath, 2000; Prieto et al., 2005;). Several measures of condition were calculated from each fish, including gonadosomatic index (GSI), gonad weight relative to eviscerated body weight (Johnston and Knight, 1999); fecundity, the number of mature ova per female estimated from a subsample following the methods of Wagner and Cooper (1963); percent (%) lipids, and Fulton's condition index, a method commonly used to analyze fish condition by calculating weight in grams relative to standard length in millimeters cubed (Hoque et al., 1998).

% Lipids

Percent lipids is of particular interest because Eckmann (2004) found when measuring many indices of condition in juvenile rainbow trout with increasing food deprivation, % lipids showed the greatest difference (Bonferroni posteriori tests after Two-Way ANOVA, $P = < 0.001$). Many different methods have been utilized to determine total lipids in fishes, but for this study, we have chosen to use the petroleum ether method employed by Heulett et al. (1995). The fish were eviscerated to remove any food items that could influence lipid results, and gonads removed from fish to calculate GSI were placed back in the body cavity. Each fish was ground up using a mortar and pestle, and a portion of the ground fish was placed in a glass vial. The vials had been labeled and weighed prior to addition of the ground fish. The vials were placed in a drying oven for 48 hours and then moved to a desiccator at 0% humidity for 48 hours. The vials were removed from the desiccator and weighed to determine dry weight. Next, each vial underwent three petroleum ether washes. Approximately 2 milliliters of petroleum ether was added to each vial, the vials were covered and allowed to sit for 45 minutes to one hour, and the petroleum ether was removed. This was repeated for each wash. The vials were placed back in the drying oven for 48 more hours and then moved to the desiccator for 48 hours. Vials were removed from the desiccator and weighed again. Pre and post extraction weights were used to determine the percent of each fish that was storage lipids.

We quantified FA as the Euclidian distance in ordination space between left and right side traits for each individual. All left traits were run in a principle components analysis (PCA) that accounted for 93% of the variability in left traits among the first two axes. Right side traits were projected onto that ordination by summing the standardized products of factor scores and measurements for each trait. FA for an individual was then quantified as the distance in two-dimen-

Fluctuating Asymmetry and Condition in Fishes Exposed to Varying Levels of Environmental Stressors

Zuber and Schaefer

sional ordination space between the left side score and the projected right side score for each individual. For example, a perfectly symmetric fish would have identical left and right side measures and the projected right side point would have a Euclidian distance of 0.0 from the left side. The more asymmetric the measures, the greater the distance between the left side and projected right side measures.

Results

The following results are from analyzing 20 *Cyprinella venusta* from the 3 km upstream site and 20 *Cyprinella venusta* from the 3 km downstream site (Figure 1).

Fluctuating Asymmetry

There was no significant difference in levels of FA measured in the fish from the 3 km upstream sites compared to fish from the 3 km downstream. Fish from the upstream site showed slightly higher levels of FA than fish from the downstream site (Figure 2). A simple linear regression model was used to detect any correlation between FA and percent lipids ($p \leq 0.84$, $r^2 = 0.001148$, FA = 0.6958664x - 0.0090148 % lipids) and between FA and index of condition ($p \leq 0.93$, $r^2 = 0.00026$, FA = 0.5935808x – 5275.2682 index of condition). Neither was significant, but both showed a slight positive correlation with percent lipids and condition increasing as levels of FA decreased.

Condition

A simple linear regression model was used to detect any correlation between percent lipids and condition ($p \leq 0.79$, $r^2 = 0.001847$, % lipids = 21.237133x + 52889.862 index of condition). There was no significant correlation, but there was a trend as condition increased slightly with an increase in percent lipids. A One-Way Analysis of Variance (ANOVA) of fecundity by site (upstream (U) mean = 243.3, downstream (D) mean = 123.1, $p \leq 0.08$) showed no significant difference in fecundity in fish collected from the downstream site compared to fish collected from the upstream site, but fish from the upstream site showed slightly higher fecundity than did fish from the downstream site. A One-Way ANOVA of GSI by site (U mean = 4.76, D mean = 2.30, $p \leq 0.01$) detected a significant difference in the GSI of fish collected from the downstream site compared to fish collected from the upstream site with fish from the upstream

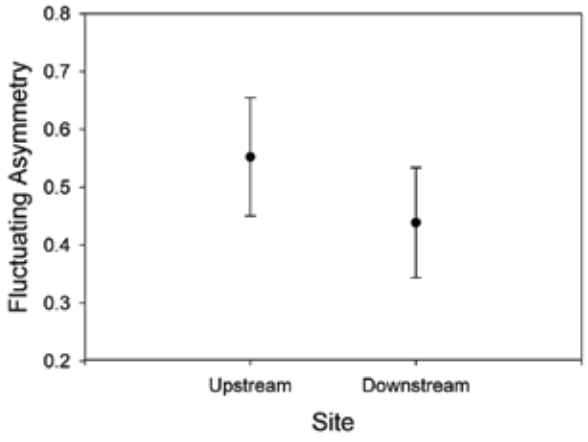

Figure 2. Mean levels of FA of fish collected from upstream and downstream sites.

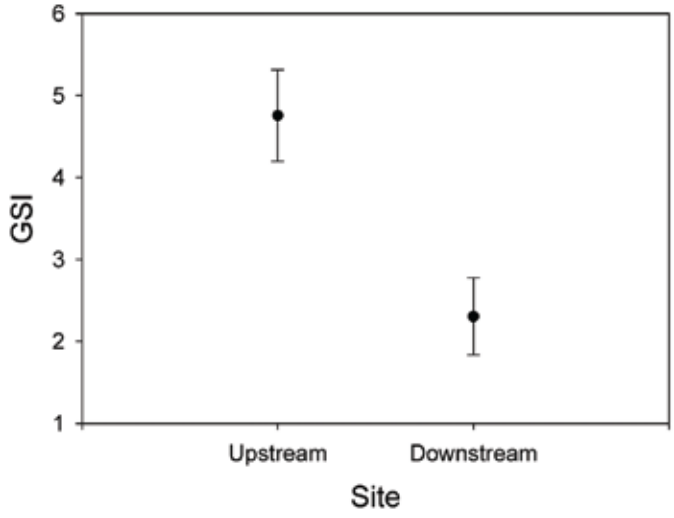

Figure 3. GSI of fish collected from upstream and downstream sites.

site showing higher GSI than fish from the downstream site (Figure 3). A One-Way ANOVA of condition by site showed no significant difference in Fulton's condition index in fish from the upstream site compared to fish from the downstream site, but condition was higher in fish from the upstream site. A One-Way ANOVA showed no difference in percent lipids by site (U mean = 22.18, D mean = 22.26, $p \leq 0.89$).

Discussion

There were no significant differences in fish collected from the upstream site compared to fish collected from the downstream site with regards to FA, fecundity, condition, or percent lipid. Levels of FA were unexpectedly higher at the upstream site. Condition and fecundity were slightly higher at the upstream site as well while percent lipid was relatively the same in fish from the upstream and downstream sites. The only significant difference occurred when analyzing GSI. GSI was found to be significantly higher in fish collected from the upstream site. FA showed no significant correlation with percent lipid or condition, but there was a trend of percent lipid and condition increasing with decreasing levels of FA. The same trend was seen in the correlation between percent lipid and condition.

The results so far are only preliminary and show little difference in fish upstream and downstream of the pulp mill, but this is only after analyzing 40 fish from one species. In the future, many more fishes will be analyzed. If 20 fish from each of the 3 species are collected from each of the 8 sites 4 times in total (20x3x8x4), approximately 1,920 fishes will be analyzed. All species will be analyzed to determine what effects, if any, movement and diet have on condition and levels of FA. The detritivore, *C. velifer*, is expected to show greater levels of FA and lower condition because of its proximity to the contaminants found in the particulate matter of the pulp mill effluent. Similarly, *L. megaloits* may also be exposed to contaminants via floating particulate matter. Home range and movement may also have an effect on condition and levels of FA. *L. megalotis* has been shown to have a small home range while *C. venusta* and *C. velifer* have larger home ranges, and their high degree of movement up and downstream may influence any impact the pulp mill effluent may have on them.

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