Spring 5-2013

Fluctuating Asymmetry of *Menidia beryllina* as a Measure of the Environmental Stress Caused by the 2010 Deepwater Horizon Oil Spill

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By

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A Thesis

Submitted to the Honors College of
The University of Southern Mississippi
in Partial Fulfillment
of the requirements for the Degree of
Bachelor of Science
in the Department of Biological Sciences

April 2013

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Abstract

Fluctuating asymmetry (FA) is often used as an indicator of environmental stress on a population. Stress encountered during development can result in asymmetries in bilateral traits. By quantifying fluctuating asymmetry in *Menidia beryllina*, FA can be used as an indicator of possible environmental stress linked to the 2010 Deepwater Horizon oil spill in the Gulf of Mexico. The hypothesis states that the levels of FA will be greater in *M. beryllina* from post oil spill samples compared to pre oil spill samples. *Menidia beryllina* were used because they are a numerically dominant species found in high wave action coastal estuaries, an area heavily affected by the oil spill. *M. beryllina* were collected monthly (beginning in May 2011) at Pascagoula River sites where specimens had been previously been collected for various pre oil spill studies. FA was then measured in three bilateral traits: eye diameter, pectoral fin length, and pelvic fin length. Data were analyzed using a two way mixed model ANOVA with side (left and right) as the fixed factor, individual and repeated measurements as factors. This model allows for measurement of FA while controlling for measurement error.

KEY WORDS: fluctuating asymmetry, oil spill, Deepwater Horizon, *Menidia beryllina*, Pascagoula River, Mississippi Sound

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Introduction

Developmental stability results from developmental processes that aid to resist stress during the development of a certain morphological trait; they are designed to counteract stress on the body to ensure that the organism will develop properly (Lens, et al., 2002). Under normal, pristine conditions, the development of larvae occurs along a pre-determined genetic path; anything that might interfere with this pathway is counteracted by a variety of physiological mechanisms (developmental stability). (Clarke, 1995). In an ideal situation, a bilaterally symmetrical organism will develop right and left sides that mirror each other; this occurs because the traits are controlled by the same gene and therefore must develop together (Klingenberg, 2003, Clarke, 1995). Stressful environmental conditions may disrupt these corrective mechanisms, which can lead to a disruption in normal developmental pathways. Fluctuating asymmetry is the most common measure of these disruptions, and is best defined as being any small, random deviations from the expected normal bilateral symmetry.

Fluctuating asymmetry (FA) is considered to be a useful bio-indicator of environmental stress on a population. Biologists have used FA to determine the fitness of a population of interest, such as fish, insects, and humans, and can assess the impact of an environmental stressor on the organisms within the population (Allenbach 2011). By looking at how symmetrical an organism is, one can determine how stable the development was. If an organism developed in an environment filled with a constant environmental stressor (chemical spills, high temperatures, etc), it can be assumed that there will be some impact on the organisms' symmetry. This approach is a well-used and test method, and can also be applied across a variety of organisms and ecosystems.

Environmental stress can affect an organism at any stage of life, but developing larvae are particularly susceptible to changes in the environment (Almeida and et. al., 2008). This can lead to an effect on developmental stability due to the present environmental stress levels (Almeida and et. al., 2008). However, when there are habitat disturbances during development that stress the larvae past the point that the body can buffer, the organism must take energy away from development and put it towards counteracting the stressor for survival (Zuber, 2008, Clarke, 1995, Lens, et al., 2002, Polak, M., et al., 2004). When energy is diverted to the stressor, the bilateral trait will develop differently from the other; any morphological change from developmental disturbance is called the developmental instability of the organism (Klingenberg, 2003, Clarke, 1995, Lens, et al., 2002).

The expected normal symmetry of traits is drawn from the developmental asymmetry that is expected for the particular organism and trait; it is rare that an organism will develop with perfect symmetry due to specific conditions that are required for it (Klingenberg, 2003, Almeida and et. al., 2008). Developmental noise refers to this non-perfect development, and is a normal occurrence due to the influences of regular cellular functions of the body (Klingenberg, 2003). Overall, FAcan be seen as the net result of developmental noise, or development instability, versus developmental stability (Vishalakshi and Singh, 2008).

Fluctuating asymmetry (FA) is commonly studied in an attempt to identify environmental stress (Polak, M., et al., 2004). This is because FA can be seen in a wide variety of different habitats, traits, and organisms; FA can also be used at several different trophic levels (Allenbach, 2011, Polak, M., et al., 2004, Zuber, 2008).

Fluctuating asymmetry is such an important area of interest because studies have shown that mate selection and survival rates are affected by the rate of fluctuating asymmetry of the individual; these effects are directly correlated with fitness of the organism (Hardersen and Frampton, 2003). Past studies have found that the level of fluctuating asymmetry has a large effect on the traits of an organism that is similar to fitness traits (Scheib, Gangestad, and Thornhill, 1999). Usually a high mating success occurs when there are high levels of symmetry as asymmetry of traits often is linked to the inability of an organism to fight off the developmental stressors that occur (Scheib, Gangestad, and Thornhill, 1999).

Fluctuating asymmetry is also commonly used because it has been found to be a useful indicator of population fitness (example studies include Polak, M., et al., 2004, Clarke, 1995, and Allenbach, 2011). For example, a study done by Hechter at al. (2000) examined the fins of female sticklebacks (*Culaea inconstans*) and found that the females with the more symmetrical fins had more eggs and bigger ovaries. One of the primary goals in conservation of a species is discovering if there is some sort of environmental stress on the species; the earlier that the stressor is found, the better chance that species has for survival (Allenbach, 2011, Leary and Allendorf, 1989). Usually the factor that affects a population most is a disturbed or polluted environment; however, by the time a species reacts to the environment, it may be too late to stop the process (Clarke, 1995, Polak, M., et al., 2004). The thought is that this phenotypical variation can be detected before there is any genetic change in the organism (Clarke, 1995). Almeida et al. (2008) examined goldfish and carp in captivity, and found that there was a useful link between environmental stressors and detectable developmental instability (Almeida and et. al., 2008)

Fishes are ideal organisms for assessing environmental stress (and FA) because they are one of the largest vertebrate groups and ubiquitous among aquatic ecosystems (Allenbach 2011). Fish also have close ties with their environment. Water is the universal solvent, so anything that gets dissolved into water can be passed directly over the gills of the fish (Allenbach, 2011).

For many decades, oil spills have been of huge concern to biologists in terms of ecological impact, especially in the marine systems (Teal and Howarth, 1984). In a review by Teal and Howarth (1984), several oil spills were compared and examined in order to determine lasting effects. One observation was that the oil in the water column can stay there for up to six months, but will usually settle to the bottom during that time period (Teal and Howarth, 1984). It was also reported that the highest level of oil hydrocarbons recorded where in high wave action areas were the oil and bottom sediment were able to mix (Teal and Howarth, 1984). *Spartina* beds, which are intertidal plants found in high wave action areas, showed persistent levels of oil six years after the *Florida* barge spill in 1969 (Teal and Howarth, 1984).

Teal and Howarth (1984) also found that even though most organisms showed signs of returning to normal after one year, the marsh ecosystem was still very heavily effected (Teal and Howarth, 1984). This study also reported that the species most effected long term by the oil were subtidal and intertidal species (Teal and Howarth, 1984). The Deepwater Horizon oil reached the marshes during the peak spawning time of many marsh organisms**.** Deepwater Horizon oil spill occurred on April 20, 2010 following an explosion at the Deepwater Horizon rig (US Department of Energy, 2011). Over the course of the next few months, the blown well spewed approximately 4.9 million barrels of oil into the Gulf of Mexico (US Department of Energy, 2011). The spilled oil ended up on beaches and in estuaries, wetlands, and marshes along the coastline of the Gulf of Mexico (US Department of Energy, 2011). Impacts on the reproductive processes of organisms from oil spills are often used as indicators to the big picture effects of the oil spill (Whitehead et al., 2011).

In terms of the organismal impacts, Teal and Howarth (1984) found that fish born during the oil spill were significantly slimmer than the control fish (Teal and Howarth, 1984). In a study involving south Louisiana crude oil effects on *Menidia beryllina*, it was found that the crude oil caused physical legions on fish (Solangi and Overstreet, 1982). These legions lead to a two third loss of olfactory senses; loss of which affected the feeding behavior of *M. beryllina*. The oil also caused hyperplasia and hypertrophy on the gills (Solangi and Overstreet, 1982). Chemoreception was altered, changing the schooling behavior and feeding habits of *M. beryllina* (Solangi and Overstreet, 1982). A more recent study done by Whitehead et al. (2011) studied the effects of the Deepwater Horizon oil spill on *Fundulus grandis* in gulf marshes (Whitehead et al., 2011). *Fundulus grandis* was chosen because of its abundance in salt marshes, a habitat Teal and Howarth (1984) found to be one of the most impacted by an oil spill (Whitehead et al., 2011). The Whitehead study (2011) found that exposure to oil did in fact directly alter patterns of gene expression.

Even though immediate oil spill effects can be seen in adult fishes, the most severe effect from the spills occurs with the fish eggs and larvae (Teal and Howarth, 1984). Solangi and Overstreet (1982) found that adult fish were able to recover from the effects of the oil spill. Teal and Howarth (1984) found that after one year adult fish were almost back to a pre-oil spill state. However, in that same study it was stated that the big problem was the damage done to eggs, larvae, and the ecosystem (Teal and Howarth, 1984). Teal and Howarth (1984) stated in their review that hatching success in the oil spill affected area was about half that are seen in an unaffected area (Teal and Howarth, 1984). Oil is considered an environmental stressor, and the developing larvae and eggs will be affected as much or worse than the adult fish. The study done by Whitehead et al. (2011) stated that the sub-lethal effects dealing with reproduction would be most likely to indicate the long term effects of the oil spill (Allenbach et al., 2011). It was also found in this study that the Gulf of Mexico is showing those early sub-lethal signals after the Deepwater Horizon oil spill (Whitehead et al., 2011).

By using fluctuating asymmetry as a tool to examine a particular fish species (*M. beryllina*), the environmental stress of the Deepwater Horizon oil spill may be measured. *Menidia beryllina* was chosen because it spawns in late April into May, and has a one or two year lifespan (Huber and Bengtson,1999). It is also abundant in high wave action coastal estuaries; areas most impacted by the oil spill (Huber and Bengtson,1999). Finally, there were numerous specimens available in ichthyological collections prior to the spill; these provide access to a baseline level of FA. Thus, the *M. beryllina* collected in Summer 2011-12 will be the fish that will have developed during the Deepwater Horizon oil spill.

My project aims to quantify fluctuating asymmetry in *Menidia beryllina* as an indicator of possible environmental stress linked to the Deepwater Horizon oil spill in the

Gulf of Mexico. The objective is to determine the levels of fluctuating asymmetry for post and pre oil spill fish in the Pascagoula River mouth. Then, using statistical analysis, determine if there is a difference between post and pre oil spill fish. I hypothesize that the levels of FA will be much greater in *Menidia beryllina* from post oil spill to pre oil spill.

Materials and Methods

The study used fish sampled from 6 different locations on the Pascagoula River, with samples collected from each site by seining or trawling once a month (see Figure 2). These sites were chosen because they had a large collection of pre-oil spill samples that were used for comparison. Having a fish for comparison is vital because in order to determine if there is any fluctuating asymmetry, the baseline level of asymmetry of the fish had to be determined. Fish for pre oil spill measures are from the ichthyology collections of The University of Southern Mississippi and the Mississippi Natural History Museum. The study was started one year after the oil spill in order to allow the generations of fish born during the oil spill to fully mature. This idea comes from Clarke (1995), and he states that when dealing with exposure time, the time exposed must be longer than the time it takes the observed generation to develop.

When choosing traits to work with, it first must be a bilateral character, and second, it must develop during the organism's exposure to the environmental stressor (Clarke, 1995). The three traits that are measured were eye diameter, pectoral fin length, and pelvic fin length. Standard length and mass were also taken in order to standardize the measurements. One of the most important components to be mindful of is measurement error; measurement error can result in a biased report of fluctuating asymmetry levels (Allenbach, 2011, Vishalakshi and Singh, 2008). Each fish was measured twice to account for measurement error, as well as separately to avoid bias.

Using life history and environmental data from Bengtson (1984) and Middaugh and Hemmer (1992), a growth curve was designed using a von Bertalanffy growth model in order to estimate birth date of each individual (Katsanevakis, 2006). Any fish that were large enough to have come from pre-2010 age classes were removed from analyses.

The statistical methods for this study were largely based on a method presented by Merilä and Björklund (1995). The raw FA data was analyzed using a two way mixed model ANOVA with the sides as the fixed factor and individuals as the random factor. From the model, a mean square value can be pulled for error and for FA. The mixed model approach allows for this separation of values; this is very important because fluctuating asymmetry values are generally small. Any measurement error present can greatly affect the FA value, resulting in false results. The FA values were reported for each trait as an overall value, as well as a broken down for pre and post oil spill (the time variable).

The ANOVA is simply being used as a computational tool to partition variance and estimate FA and measurement error. The measurement error is from the mean square within value to account for random variation within the individuals. The individual is nested within time, and is also used as the random factor. The sides (left and right) are the fixed factors. A maximum sample of n=20 fish from each site were chosen at random for measurements.

To test the hypothesis that FA values were different between pre and post-spill samples, I used a chi-squared test to compare the full model (with time as a variable) to a model without the time variable. Significance of the chi-squared test, along with a lower AIC value for models with the time variable indicated FA values differed between pre and post samples after controlling for measurement error. An alpha of 0.05 was used for significance, with values falling below the alpha to be significant.

For visualization, composite FA scores were calculated for each individual. Composite scores consist of the raw FA data (size corrected difference between right and left measures for each trait of the three traits summed. Composite scores do not account for measurement error, but allow for a single measure of individual FA. A standard ANOVA (using time as the factor) was run on pre versus post samples in order to determine significance.

Results

The total sample size was 883 total individual fish for pre and post samples, and n=724 for post oil spill fish and pre-oil spill fish n=159. The eye had an overall FA of 0.04622, the pectoral fins an overall FA of 0.10710, and for the pelvic fins 0.06235 (see Table 1). Measurement error overall for the traits was between 5-10%.

Eye FA was significantly (X^2=93.2241, df=1, p<0.0001) higher in post sample (0.05089, measurement error=0.00116) compared to pre samples (0.02511, measurement error=0.00047) (Table 2, Figure 1).

Pectoral fin FA was significantly (X^2=85.704, df=1, p=0.0002) higher in pre sample (0.13573, measurement error=0.00086) compared to post samples (0.10091, measurement error=0.00499) (Table 2, Figure 1).

Pelvic fin FA was significantly (X^2=14.116, df=1, p<0.0001) higher in post sample (0.07651, measurement error=0.02398) compared to pre samples (0.06014, measurement error=0.00316) (Table 2, Figure 1).

The composite FA scores were significantly ($F_{1,881}$=15.7, p=0.01933) higher in post spill (0.0749 +/- 0.0698 SE) than pre oil spill (-0.2883 +/- 0.1423 SE)

Discussion

The fluctuating asymmetry levels of the *Menidia beryllina* may provide some insight on the stress levels of the fish caused by the Deepwater Horizon oil spill. Two of the traits measured showed a significantly greater FA in post oil spill versus pre oil spill samples. These differences occurred in the eye diameter and the pelvic fin length. The most significant trait that showed change was the eye diameter while the pectoral fins showed the opposite trend.

It has been suggested that different traits have different tolerances to levels of stress, making some better at buffering external stressors (Vishalaksi and Singh, 2008). Vishalaksi and Singh (2008), noted an association between a traits developmental stability and its function. Traits directly related to locomotion have, over time, developed strong developmental stability due to strong selective pressure in order to counteract stressors to maximize fitness. Pectoral fins are used by fish to create lift when swimming, and variation from baseline asymmetry could significantly affect the function of the fin. *Menidia beryllina* are planktivores, actively swimming in order to find and consume food; any serious impact on the fins could hinder the organism's ability to eat, leading to a domino effect on other physiological processes (Bengston, 1984).

An alternative explanation for this reverse pattern may simply be due to the improper sorting and storage of the specimens (both pre and post). The pectoral fins are the most exposed trait to breakage during handling, and consequently had higher breakage. Fish were checked for breakage prior to measurements, but because FA is such a small value, even breakages small enough to remain unseen can cause this trend to occur.

The hydrocarbons in oil infiltrate organisms and cause not only external harm, but directly can affect the gene expression of some organisms. Whitehead et al., (2011) finds that primary toxic components of crude oil interact with receptor pathways to induce genomic expression responses in fish (Whitehead et al., 2011). The study showed the interactions of these toxins in developing fish embryos induced developmental abnormalities (Whitehead et al., 2011). The data backs this finding up in that fluctuating asymmetry (a developmental abnormality) is present in the eye and pelvic fins. However, there is not a lot of literature that has determined if the alteration of gene expression is hereditary or not; fluctuating asymmetry has support to be confined to the individual and not genetically inherited. However, several studies have been done that indicate oil is toxic to *M. beryllina*, including studies in Kuwait (Al-Yakoob et al., 1996 and Gunderson et al., 1996), Alaska (Middaugh et al., 1996), and in the Gulf of Mexico (Solangi and Overstreet, 1982).

Overall, the data indicates that there was a high enough level of environmental stress from the 2010 Deepwater Horizon spill to be detectable and cause future concerns. The statistical analysis was a combination of several theories from papers in order to account for and compensate for measurement error. Measurement error is commonly looked over or forgotten, and it can be credited with the ability to skew results. For this study, it was partitioned out of the data completely to give a confident fluctuating asymmetry score. This study also had a baseline other than zero with which to compare fluctuating asymmetry scores to. Normally, asymmetry is compared to this baseline of zero; however, because it is defined as 'any small, random deviation from normal', it is important determine first what normal is. By having the pre oil spill samples available, the baseline could be determined. For the study, it gave more power to the data to accurately show if there was an increase or decrease in the fluctuating asymmetry over the oil spill exposure time.

The study also quantified significance; this allowed for a true comparison between fluctuating asymmetry levels. By comparing the model with time (pre versus post) and without time, it allowed for a chance to truly see if what our data was telling us was significant or not, and if so, how much. With all of the model comparisons showing significance, I can conclude that the differences between pre and post-oil spill samples were significant (even if one trend was reversed)

Another way to look at the significance of the pre versus post oil spill FA levels is by simply examining the composite FA scores. With a p=0.0099, I can conclude that there is a significant difference between overall total fluctuating asymmetry from pre-oil spill samples to post-oil spill samples.

The objective of this study was to determine to determine the levels of fluctuating asymmetry for post and pre oil spill fish in the Pascagoula River mouth with the hypothesis that levels of fluctuating asymmetry would be higher in post oil spill samples compared to pre oil spill samples. The data found supports this hypothesis in that the oil spill did cause an increase in the FA levels of *Menidia beryllina* in the lower Pascagoula River. This finding also adds support to the idea that, when done correctly, fluctuating asymmetry can prove to be a useful tool when determining levels of environmental stress in ecosystems. For future work, taking a look at other species in the same environment would prove useful in order to give a broader picture to the impacts of the oil spill. This could include studying invertebrates (crabs or shrimp), as well as other species fish. Perhaps looking at other traits that are not as strongly correlated with fitness could help support this hypothesis even more. The one trait that did not support the hypothesis, the pectoral fin, is a key component to the individual's fitness and has been strongly selected for developmental stability. Traits such as otolith lengths and widths, along with lateral scale counts, could be useful traits to add to the study.

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Table 1: Pooled overall fluctuating asymmetry (FA), measurement error (ME), and percent measurement error (%ME) measurement error for pre and post oil spill samples.

n=883	FA	ME	% ME
Eye	0.04622	0.00104	2.24%
Pectoral fin	0.10710	0.00425	3.97%
Pelvic fin	0.06235	0.00601	9.65%

Table 2: Overall fluctuating asymmetry (FA), measurement error (ME), and percent measurement error (%ME) for each trait for pre and post oil spill samples.

n=159, 724	FA	ME	%ME
Eye			
Pre	0.02511	0.00047	1.86%
Post	0.05089	0.00116	2.28%
Pectoral fin			
Pre	0.13573	0.00086	0.64%
Post	0.10091	0.00499	4.95%
Pelvic fin			
Pre	0.06014	0.00316	5.26%
Post	0.07651	0.02398	31.34%

Table 3: Composite means for raw fluctuating asymmetry data for pre and post samples, along with the standard error values

	Post	Pre
Mean	0.07486	-0.28832
Standard Error	0.0698	0.14227

Figure 1: Composite FA values for each trait plotted to show differences between pre and post spill values. Error bars indicate standard error. There is overlap in the pectoral fin because measurement error is not accounted for in this figure

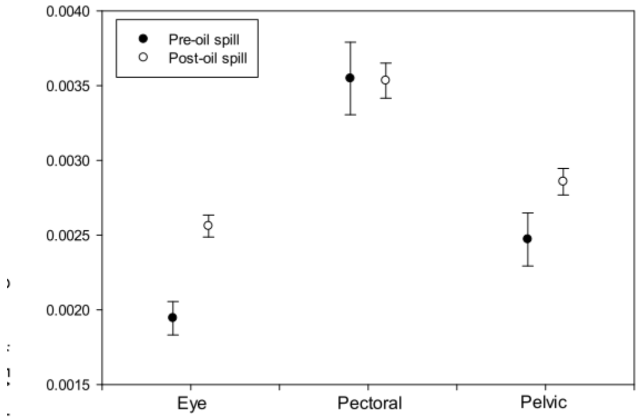

Figure 2: Map of oil spill area with Pascagoula Sites indicated with yellow markers