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A

A THREE-PARAMETER DEVELOPMENTAL MODEL FOR ANALYZING FLUCTUATING ASYMMETRY IN MERISTIC CHARACTERS

by

JOHN RAYMOND YOUNG

A dissertation submitted to the Graduate Faculty in Biology in partial fulfillment of the requirements for the degree of Doctor of Philosophy, The City University of New York.

1999

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This manuscript has been read and accepted for the Graduate Faculty in Biology in satisfaction of the dissertation requirement for the degree of Doctor of Philosophy.

December 7, 1998
Date

Chair of Examining Committee
Dr. Robert F. Rockwell, City College

January 13, 1999
Date

Richard L. Chappell
Executive Officer
Dr. Richard L. Chappell

John H Tietjen
Dr. John Tietjen, City College

John Waldman
Dr. John Waldman, Hudson River Foundation

Ernest Lorda
Dr. Ernesto Lorda, Northeast Utilities Service Company

Wm L Kirk
Dr. William Kirk, Consolidated Edison

Supervising Committee

The City University of New York

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Abstract

A THREE-PARAMETER DEVELOPMENTAL MODEL FOR ANALYZING FLUCTUATING ASYMMETRY IN MERISTIC CHARACTERS

by

John Raymond Young

Adviser: Professor Robert Rockwell

Fluctuating asymmetry has been proposed as a population index of developmental stress resulting from pollution, extreme genetic homozygosity or heterozygosity, or environmental conditions. However, in many instances, measures of fluctuating asymmetry in meristic characters have been inconsistent in distinguishing among populations exposed to different levels of contaminants or with different amounts of genetic variation. An alternate measure of fluctuating asymmetry, $\hat{\sigma}_a$, is derived from a three-parameter model of the developmental process. A numerical search algorithm is used to find maximum likelihood estimates of the model parameters μ, σ_L, and σ_a.

$\hat{\sigma}_a$ is less sensitive to bias from variation in the mean character value than are indices based on the difference between right and left counts. Likelihood ratio tests for $\hat{\sigma}_a$ exhibit nominal levels of α-error, and yet are as or more powerful than tests for the alternative measures.

Data from five studies of fluctuating asymmetery in fish were reanalyzed using $\hat{\sigma}_a$ and compared to the analyses

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originally reported. Overall, use of the three-parameter model did not change the conclusions of the original investigators. The failure to find strong relationships of heterozygosity and pH with fluctuating asymmetry could be due to a truly weak relationship, or to other factors such as low sample sizes, errors in character counts, or use of mixed-age samples.

Fluctuating asymmetry of pectoral fin rays was also examined in anadromous striped bass collected in from the Roanoke River, Chesapeake Bay, and Hudson River in 1974-1975 and 1989. Fluctuating asymmetry was estimated by ∂_a and three additional measures. Tests based on ∂_a indicated significant differences among the three stocks, and between the two data sets for each of the three stocks. Tests based on the other three asymmetry indices were not as powerful at detecting these differences.

When the data were segregated by year class and subjected to a nested ANOVA, year-within-stock-and-river was the only variance component that exceeded the magnitude of the error component, indicating that year-to-year variation in fluctuating asymmetry is large for these populations and could obscure any differences due to stock, or river within stock.

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ACKNOWLEDGMENTS

There have been many people who have influenced and encouraged me during the course of my studies and in the completion of this dissertation. First, my advisor, Dr. Robert Rockwell has provided guidance and encouragement through the process. Dr. John Waldman has also contributed ideas and patience as we worked slowly toward the conclusion of this research. The other members of the committee, Drs. William Kirk, John Tietjen, and Ernest Lorda also were helpful in improving the dissertation and guiding my course of study.

In this work, I have used a great deal of data collected originally by other researchers. Without exception they were very supportive and willingly contributed their data to be used for testing the analytical techniques that are the subject of this research. For that I thank Paul Rago, Joe Quattro, Charles Jagoe, Rob Leary, Fred Allendorf, and Mike Bell, whose data were collected under grant BSR-8905758.

The striped bass data of Chapter 4 were collected in two separate investigations. For the 1974-1975 data, I a thank the many fine people I worked with at Texas Instruments Environmental Services Division. The 1989 data were collected in a study directed by John Waldman with assistance from L. Blumberg, M. Chang, S. Early, K. Hattala, A. Kahnle, J. Loesch, A. Loftus, J. Morales, I. Wirgin, and D. Yozzo for their help in obtaining the 1989 samples. We

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are also grateful to B. Young and V. Vecchio for determining the age of specimens collected during 1989 and to J. Hamilton for assistance in data analysis.

I also thank my employer, Consolidated Edison of New York, for financial assistance, for use of their data, for allowing me the flexibility to complete this program, and for encouragement.

Finally, I thank my wife Deborah, and children Erin, Joshua, Emma, and Sara for being understanding when I had to borrow time that would otherwise have been spent on family to go to class, study, or work on my research. Without their patience and understanding, this would not have been possible.

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CHAPTER 1

DEVELOPMENTAL INSTABILITY AS AN INDICATOR OF STRESS

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INTRODUCTION

The study of genetic and environmental influences on the development of the phenotype, now known as phenogenetics (Zakharov & Graham 1992a), has been going on for over seventy years since Sumner and Huestis (1921, cited in Angus 1982) related developmental instability to genetic factors. Although intensive studies of the stability of development were done in the early part of this century, e.g. Astauroff (1930, cited in Zakharov and Graham 1992a), widespread interest in the topic took longer to develop. Waddington, through his work in the 1940s and 1950s introduced the concepts of canalization and developmental stability (Zakharov and Graham 1992a). Canalization is stability of development in different environments, while developmental stability denotes the repeatability of development in the same environment. Much of the pioneering work in the field occurred in the 1950s and early 1960s through laboratory experiments on *Drosophila* (Mather 1953; Tebb and Thoday 1954; Thoday 1955; Reeve 1960; Parsons 1962). Van Valen (1962) provided a framework for the use of asymmetry in quantifying instability. Once the concepts were developed, extension to wild populations was pioneered by Soulé (1967).

In the last 30 years, interest in phenogenetics has grown substantially and it has been applied in a wide range of circumstances to many different organisms. Several international symposia have been held and the proceedings have been published to advance research in the field

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(Zakharov and Graham 1992b; Markow 1994). Through all of the advances in the field, a key difficulty in analysis of meristic characters (Swain 1987), which results in a variable degree of bias in certain situations, has remained unsolved (Session 1, Measuring Developmental Instability,in Markow 1994). The aim of this research is to provide a solution to the analysis problem and thereby advance the use of meristic characters in phenogenetic studies.

SYSTEMATIC ANALYSIS OF ASYMMETRY

Van Valen (1962) classified bilateral asymmetry into three different types; directional asymmetry, antisymmetry, and fluctuating asymmetry. Directional asymmetry occurs when one side of an organism is universally different than the other side, as occurs in the flatfishes in which the two sides have extreme morphological differences. Antisymmetry occurs when the side with the larger structure is random. The example usually given for antisymmetry is the claw asymmetry that occurs in male fiddler crabs (Uca musica). Fluctuating asymmetry, the type most associated with developmental instability, occurs when the usual condition is bilateral symmetry, but one side or the other can at times have a larger structure, or one with a greater count for meristic characters.

These three types of asymmetry are usually easily distinguished by the statistical properties of the difference between the bilateral structures. In directional

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asymmmetry, the difference will have a normal distribution, but the mean value will be decidedly different from zero. For antisymmetry, the mean will be zero, but the distribution of differences will be bi-modal. In fluctuating asymmetry, the single mode of the distribution will be zero and the dispersion will be that of the normal distribution.

Thus the usual means of distinguishing the three types of asymmetry are to test the distribution of differences for a mean equal to zero, and for significant skewness and kurtosis (Palmer 1994). Directional asymmetry is indicated by a mean different from zero, antisymmetry by significant platykurtosis, and fluctuating asymmetry by no significant result for any of these three tests.

PROXIMAL CAUSES

In spite of all the years of study of developmental stability, the exact mechanisms or pathways that are disrupted by stress is unknown. Freeman et al. (1993) have suggested that stress disrupts the fine-tuned metabolic pathways of cell growth. The kinetics of enzymes and substrate reactions must be closely matched for development to proceed properly. Any change in availability of an enzyme at the place and time it is needed can disrupt the complex chain of reactions that comprise the growth process.

Disruptions of the growth process tend to be magnified in a non-linear fashion (Emlen et al. 1993). In normal

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development there must be mechanisms for cell-cell feedback that allow organisms to correct minor deviations in development that will inevitably occur. Feedback must occur both among the neighboring cells within a morphological feature, and between the cells of the same feature on the two sides of an organism. Emlen et al. (1993) suggested that the feedback tends to create phase-locking of growth on the two sides of an organisms, such that growth may proceed in pulses alternating from one side then the other. Any disruption that would change the strength or time-lag of the feedback signal could adversely affect phase-locking and lead to developmental instability, resulting in one of the three types of asymmetry (Graham et al. 1993a). They conclude that some asymmetry may simply be the result of the normal indeterminate nature of the non-linear growth process and may not indicate developmental disruption at all. In addition, they suggest that both directional asymmetry and antisymmetry, like fluctuating asymmetry, may be the result of growth process disturbances.

ULTIMATE CAUSES

Nilsson (1994), who observed that European nuthatches (Sitta europaea) showed higher asymmetry in rectrices formed during periods of low food availability, concluded that energetic stress is the ultimate cause of asymmetry.

The two most commonly examined causes of developmental instability are contaminant stress and genetic stress.

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Contaminant stress can result from exposure of the developing organism to contaminants in the external environment, or to contaminants in it's internal environment prior to birth or hatching. The sensitivity of embryonic organisms to low levels of contaminants has recently been the subject of a great deal of study (Colborn et al. 1995).

The source of genetic stress has been ascribed to both low heterozygosity (due to inbreeding) and disruption of coadapted gene complexes (due to inbreeding or outbreeding). Although most of the earlier papers focused on low heterozygosity as the source of genetic stress (Soulé 1979; Felley 1980; Vrijenhoek and Lerman 1982; Quattro and Vrijenhoek 1989), more recent work has suggested disruption of coadaption as the ultimate reason for genetic stress (Graham and Felley 1985; Graham 1992; Clark 1994)

APPLICATION OF FLUCTUATING ASYMMETRY

Even without having an exact physiological and biochemical explanation for asymmetric growth, fluctuating asymmetry may still be very useful as an indicator of a population under stress. Graham et al. (1993a, 1993b) describe how the phenomenon is useful in the field of ecotoxicology as a way to detect populations that are undergoing contaminant stress. They also suggest ways to examine developmental disruptions in species that are not bilaterally symmetrical. The application to plant species and ecosystems was also suggested by Tracy et al.(1995) and

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Freeman et al.(1995). Leary and Allendorf (1989) demonstrate the uses for fluctuating asymmetry in the field of conservation biology, while Palmer (1986) applied fluctuating asymmetry to paleontology.

FLUCTUATING ASYMMETRY AND FITNESS

Developmental instability is valuable as a measure of stress because, not only is it an easily measured early warning of stress, it can also indicate more serious effects that are already occurring. The evidence for this is that there are many instances in which asymmetry co-occurs with reduced fitness (Møller 1997, Clark 1995). Fluctuating asymmetry has been linked to reduced survival or fecundity (Quattro and Vrijenhoek 1989). Moran et al.(1997) found that the asymmetry level in cohorts of Atlantic salmon (Salmo salar) decreased through time, indicating lower survival of asymmetric individuals. Asymmetry can also be a trait acted upon by sexual selection that effectively reduces asymmetric individuals' chance of passing on their genes to the next generation (Rohde et al.1997, Watson and Thornhill 1994).

FLUCTUATING ASYMMETRY IN FISHES

Fishes seem to be prime candidates for the use of fluctuating asymmetry as a measure of developmental instability. Fishes, to a greater extent than most other vertebrate groups, have external bilateral structures that

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have countable elements that have long been used for purposes of identifying species. These structures (fin rays, scales, pores) are relatively easily counted, even in situations where the specimens are to be kept alive, and seem to have some inherent level of variability, which indicates that the character value is not closely tied to fitness. In any case, no specialized preparation or equipment is usually required, thus fluctuating asymmetry of meristic characters could be a quick and inexpensive way to assess stress, and most studies of fluctuating asymmetry in fish have used primarily meristic characters.

Although asymmetry had been a topic of interest to ichthyologists for some time (Hubbs and Hubbs 1945), the study of fluctuating asymmetry on fishes dates back only to 1973. Valentine and Soulé (1973) obtained grunion (Leuresthes tenuis) eggs spawned in the wild, moved them to the laboratory, exposed the developing embryos to different concentrations of DDT, and demonstrated that higher concentrations of DDT resulted in higher fluctuating asymmetry. They also (Valentine et al. 1973) were the first to apply fluctuating asymmetry analysis to wild populations and were able to demonstrate a trend of increasing asymmetry with proximity to major pollution sources on the Pacific coast.

After the success of Valentine and Soulé (1973) and Valentine et al. (1973), fluctuating asymmetry appeared to be a promising tool for examining pollution stress in wild

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populations. Unfortunately, results of subsequent studies were not as clearcut. Ames et al. (1979) attempted to find fluctuating asymmetry differences in largemouth bass (Micropterus salmoides), bluegills (Lepomis macrochirus) and redbreast sunfish (L. auritus) in various ponds with and without addition of heated effluents. Although they were unsuccessful in finding differences in fluctuating asymmetry as a result of the added heat, the did find that mercury pollution was associated with higher asymmetry. The mixed results of Ames et al. (1979), were followed by similar equivocal studies. Heitzman (1982), in the only other experimental study manipulating exposures to contaminants in fish, found that fluctuating asymmetry of lateral line scales, scale rows, and pectoral fin rays increased when fathead minnow (Pimephales promelas) eggs were exposed to 100 μg/L PCBs, but no effects were found for 5 μg/L DDT or $HgCl_2$. Carline and Lawal (1985) concluded that fluctuating asymmetry of pectoral fin rays was not a useful method of detecting pollution stress in Ohio and Wisconsin populations of yellow perch (Perca flavescens). Jagoe and Haines (1985), and Weiner and Rago (1987) attempted to relate fluctuating asymmetry to pH of the environment. Jagoe and Haines found that some characters of brook trout (Salvelinus fontinalis) had higher levels of fluctuating asymmetry in lakes with lower pH, but white suckers (Catostomus commersoni) and lake chub (Coesius plumbeus) showed little association between asymmetry and pH.

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During the 1980s, fluctuating asymmetry was also examined as an indicator of genetic stress for numerous populations, with a variable degree of success. Felley (1980) found that hybrid bluegill populations in the southeast United States levels of asymmetry similar to those of pure populations outside the hybrid zone. However, five years later Graham and Felley (1985) were able to detect higher fluctuating asymmetry (indicating genetic stress) among hybrids between two species of Enneacanthus in New Jersey. Other examinations of genetic stress included Vrijenhoek and Lerman (1982), in which small local populations of Poceiliopsis monacha, with low heterozygosity, were no more asymmetric than larger, more heterozygous, populations. In contrast, Quattro and Vrijenhoek (1989) found that relict populations of Sonoran topminnow (P. occidentalis) were increasingly asymmetric as heterozygosity declined.

The interest of scientists from the Soviet Union also became apparent as Zakharov and Zyuganov (1980) related fluctuating asymmetry to heterozygosity at two loci that determine lateral plate morphs in threespine sticklebacks (Gasterosteus aculeatus). The Soviet scientists had their own predecessors in the use of asymmetry since Astauroff (1930) had previously done considerable work in this area. However, this early Soviet work is still not widely known or available to western scientists.

The mixed results with fish populations was similar to

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that with many other organisms. Palmer and Strobeck (1992) suggested that the inconsistent results of fluctuating asymmetry studies was at least partly attributable to the wide variety of statistics and hypothesis tests that have been used. In many cases, the fluctuating asymmetry metrics employed were inappropriate and tests were not optimal. They suggested ways to standardize the analysis of morphometric asymmetry data so that greater comparability would be achieved between studies.

METHODOLOGY OF FLUCTUATING ASYMMETRY

Although there has been considerable attention to the statistical methods used to analyze asymmetry data, the thrust of most of the published work has been to use the difference of the measurement or count as the observation, and to employ techniques based on normality of the difference distribution (Palmer and Strobeck 1992), or to use non-parametric techniques that do not assume normality. This emphasis on normal-theory statistics has arisen from the conceptual developmental model originally proposed by Van Valen (1962) and further elaborated for meristic characters by Swain (1987). Most studies have used the difference as the basic datum, but in anthropology at least, the correlation between the underlying right and left values was apparently in favor with some investigators, prompting Angus (1982) to demonstrate the problems with using correlation (or lack of) as a measure of asymmetry. Palmer

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(1994) provides a thorough review and explanation of analysis methods.

Discussion of appropriate methodology is ongoing. Swaddle et al. (1994), Simmons et al. (1995), Pomoroy (1997), Swaddle (1997), Thomas and Poulin (1997), Graham et al. (1998), and Gangestad and Thornhill (1998) have all made recent contributions to the analysis of fluctuating asymmetry.

In spite of these advances, none of these papers have considered the inherent difference between morphometric and meristic data, and therefore no solution to the problem described by Swain (1987) has been found (Markow 1994).

The analysis problem arises from the nature of meristic characters, in which they are considered to arise from an underlying normal distribution termed the "liability." The liability contains threshold values which serve to divide the continuous liability into discrete integer-valued observations. Developmental instability can cause a deviation from the mean which, if sufficient to cause the observed liability to cross one of the thresholds, will result in a deviation from the typical meristic count for the character. The developmental deviations on each side of the organism are considered to be independently normally distributed with a mean of zero and a common variance. Depending on the mean value of the liability for a particular population, a given magnitude of deviation may or may not cause the observed liability to cross a threshold.

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For example, if the mean is an integer value and the thresholds are midway between integers, then a deviation must exceed +/-0.5 in order to affect the character count. However, if the mean is near a threshold, then a smaller deviation in the direction of the nearest threshold will be suffient to affect the character count. Populations whose mean is centered between two thresholds will be relatively insensitive to developmental anomalies, while a population whose mean is near a threshold will show more asymmetry for the same amount of instability. This "Swain phenomenon" may partly explain why fish studies, which rely primarily on meristic characters, have been so inconsistent in demonstrating positive results.

FLUCTUATING ASYMMETRY THEORY

Until very recently, the original description of fluctuating asymmetry by Van Valen (1962) has formed the theoretical basis for most studies until very recently. Under this conceptual model, the difference in count or measurement between right and left sides would form a normal distribution, and the amount of developmental instability would be indicated by the variance of the differences. Swain (1987) explained this underlying conceptual model of development using two parameters, the mean of the liability, and the variance resulting from developmental instability. This model provides a normal distribution for the character values and, since the difference of two normally distributed

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variates is also normal, a normal distribution for the differences.

This simple conceptual model of character development has been the essentially unchallenged until recently. Emlen et al. (1993) proposed a nonlinear growth dynamics model that could account not only for fluctuating asymmetry, but also directional asymmetry and antisymmetry. They followed this with a model of dynamic morphogenesis that may also have broad applicability (Graham et al. 1993a). Leung and Forbes (1997) recently proposed a model in which fluctuating asymmetry can be explicitly related to stress and fitness.

However, none of these new models have addressed the "Swain problem", nor have the models been presented so that the model parameters can be estimated from empirical data. Thus they may be useful in guiding further theoretical development, but they are not at this time able to guide statistical analysis.

The methods presented in Chapter 2, and employed in Chapters 3 and 4, were developed expressly to remove the variable bias in fluctuating asymmetry estimates due to the location of the mean of the liability. The model proposed builds upon the work of Van Valen and Swain by adding one additional parameter and providing the mathematical methods (maximum likelihood and gradient search) to estimate the model parameters from real data. The additional parameter provides for random changes in the man liability among organisms within a population. Swain (1987) actually

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presented a simpler version of a three-parameter model in which the mean liability was determined by a singel genetic locus with two alleles. The statistical methods also include likelihood ratio tests to allow a comparison of the new model with the simpler model from Van Valen and Swain.

Chapters 2, 3, and 4 have been written as stand-alone manuscripts that will be submitted to scientific journals upon completion of this dissertation, therefore each chapter contains its own abstract, literature cited, and standard subsections of a scientific manuscript. Chapter 2, authored by J. R. Young, will be submitted to the Journal of Agricultural, Bioligical and Environmental Statistics. Chapter 3, coauthored with J. R. Waldman, and Chapter 4, coauthored with J. R. Waldman and T. B. Hoff, will be submitted to the Transactions of the American Fisheries Society.

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REFERENCES

Ames, L. J., J. D. Felley, and M. H. Smith. 1979. Amounts of asymmetry in centrarchid fish inhabiting heated and nonheated reservoirs. Transactions of the American Fisheries Society 108:489-495.

Angus, R. A. 1982. Quantifying fluctuating asymmetry: Not all methods are equivalent. Growth 46:337-342.

Astauroff, B. L. 1930. Analyse der erblichen Störungsfälle der bilateralen Symmetrie im Zusammenghang mit der selbständigen Variabilität ähnlicher Strukturen. Zeitschr. Indukt. Abst. Vererb. 55:183-262.

Carline, R. F. and M. V. Lawal. 1985. Contaminants and bilateral asymmetry in yellow perch. Environmental Toxicology and Chemistry 4:543-547.

Clarke, G. M. 1994. The genetic basis of developmental stability. I. Relationships between stability, heterozygosity and genomic coadaptation. Pages 17-25 In T. A. Markow (ed.) Developmental Instability: Its Origins and Evolutionary Implications. Kluwer Academic Publishers. Netherlands.

Clark, G. M. 1995. Relationships between developmental stability and fitness: Applicaton for conservation biology. Conservation Biology 9(1):18-24.

Emlen, J. M., D. C. Freeman and J. H. Graham. 1993. Nonlinear growth dynamics and the origin of fluctuating asymmetry. Genetica 89:77-96.

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Escós, J., C. L. Alados, J. M. Emlen, and S. Alderstein. 1995. Developmental instability in the Pacific hake parasitized by Myxosporeans Kudoa spp. Transactions of the American Fisheries Society 124:943-945.

Felley, J. 1980. Analysis of morphology and asymmetry in bluegill sunfish (Lepomis macrochirus) in the southeastern United States. Copeia 1980(1), pp.18-29.

Freeman, D. C., J. H. Graham, and J. M. Emlen. 1993. Developmental stability in plants: symmetries, stress and epigenesis. Genetica 89:97-119.

Freeman, D. C., J. M. Emlen, J. H. Graham, R. L. Mara, M. Tracy, and C. L. Alados. 1995. Developmental instability as a bioindicator of ecosystem health. Pages 170-177. In Proceedings: shrubland ecosystem dynamics in a changing envirnment.

Graham, J. H. and J. D. Felley. 1985. Genomic coadaptation adn develoopmental stability within introgressed popuatoins of Enneacanthus gloriosus and E. obesus (Pisces, Centrarchidae). Evolution 39:104-114.

Graham, J. H. 1992. Genomic coadaptation and developmental stability in hybrid zones. Acta Zoologica Fennica 191:121-131.

Graham, J. H., D. C. Freeman, and J. M. Emlen. 1993. Developmental Stability: A sensitive indicator of populations under stress. Pages 136-158 In Landis, W. G., J. S. Hughes, and M. A. Lewis (eds.) Environmental Toxicology and Risk Assessment, ASTM STP 1179.

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Philadelphia.

Graham, J. H., J. M. Emlen, and D. C. Freeman. 1993a. Developmental stability and its applications in ecotoxicology. Ecotoxicology 2:175-184.

Graham, J. H., D. C. Freeman and J. M. Emlen. 1993b. Antisymmetry, directional asymmetry, and dynamic morphogenesis. Genetica 89:121-137.

Graham, J. H., J. M. Emlen, D. C. Freeman, L. J. Leamy, and J. A. Kieser. 1998. Directional asymmetry and the measurement of developmental instability. Journal of the Linnean Society 64:1-16.

Heitzman, R. A. 1982. DDT, Aroclor 1254, and mercuric chloride as potential inducers of bilateral asymmetry in the fathead minnow (_Pimephales promelas_). M. S. Thesis. The Ohio State University, Columbus.

Hubbs, C. L. and L. C. Hubbs. 1945. Bilateral asymmetry and bilateral varaiation in fishes. Papers form the Michigan Academ of Science, Arts and Letters 30:229-311.

Jagoe, C. H. and T. A. Haines. 1985. Fluctuating asymmetry in fishes inhabiting acidified and unacidified lakes. Canadian Journal of Zoology 63:130-138.

Leary, R. F. and F. W. Allendorf. 1989. Fluctuating asymmetry as an indicator of stress: implications for conservaton biology. Trends in Ecology and Evolution 4:214-217.

Leung, B. and M. R. Forbes. 1997. Modelling fluctuating

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asymmetry in relation to stress and fitness. Oikos 78:397-405.

Mather, K. 1953. Genetical control of stability in develpoment. Heredity 7:297-336.

Markow, T. A. 1994. Developmental Instability: Its Origins and Evolutionary Implications. Kluwer Academic Publishers. Netherlands.

Møller, A. P. 1997. Developmental stability and fitness: a review. The American Naturalist 149:916-932.

Moran, P, J. I. Izquierdo, A. M. Pendas, and E. Garcia-Vazquez. 1997. Fluctuating asymmetry and isozyme variation in Atlantic salmon: Relation to age of wild and hatchery fish. Transactions of the American Fisheries Society 126:194-199.

Nilsson, J. 1994. Energetic stress and the degree of fluctuating asymmetry: implications for a long-lasting, honest signal. Evolutionary Ecology 8:248-255.

Palmer, A. R. and C. Strobeck. 1992. Fluctuating asymmetry as a measure of develpmental stability: Implications of non-normal distributions and power of statistical tests. Acta Zoologica Fennica 191:55-70.

Palmer, A. R. 1986. Inferring relative levels of genetic variability in fossils: the link between heterozygosity and fluctuating asymmtery. Paleobiology 12(1):1-5.

Palmer, A. R. 1994. Fluctuating asymmetry analyses: A

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primer. Pages 335-364 In T. A. Markow (ed.), Developmental Instability: Its Origins and Evolutionary Implications. Kluwer, Dordrecht, Netherlands.

Parsons, P. A. 1962. Maternal age and developmental variability. Journal of Experimental Biology 39:251-260.

Pomory, C. M. 1997. Fluctuating asymmetry: biological relevance or statistical noise. Animal Behavior 53:225-227.

Quattro, J. M. and R. C. Vrijenhoek. 1989. Fitness differences among remnant populations of the endangered Sonoran topminnow. Science 245:976-978.

Reeve, E. C. R. 1960. Some genetic tests on asymmetry of sternopleural chaeta number in Drosophila. Genetic Research 1:151-172.

Rohde, P. A., T. Amundsen and P. Fiske. 1997. Fluctuating asymmetry, mate choice and experimental designs. Animal Behavior 54:1030-1033.

Simmons, L. W., J. L. Tomkins and J. T. Manning. 1995. Sampling bias and fluctuating asymmetry. Animal Behavior 49:1697-1699.

Soulé, M. E. 1967. Phenetics of natural populations. II. Asymmetry and evolution in a lizard. American Naturalist 101:141-160.

Soulé, M. E. 1979. Heterozygosity and developmental stability: Another look. Evolution 33:396-401.

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Sumner, J. L. and R. R. Huestis. 1921. Bilateral asymmetry and its relation to certain problems of genetics. Genetics 6:445-485.

Swaddle, J. P. 1997. Experimental design and signalling properties of fluctuating asymmetry. 54:1034-1037.

Swaddle, J. P., M. S. Witter and I. C. Cuthill. 1994. The analysis of fluctuating asymmetry. Animal Behavior 48:986-989.

Swain, M. W. 1987. A problem with the use of meristic characters to estimate developmental stability. The American Naturalist 129:761-768.

Tebb, G. and J. M. Thoday. 1954. Stability in development and relational balance of X-chromosomes in Drosophila melanogaster. Nature 174:1109-1110.

Thoday, J. M. 1955. Balance, heterozygosity and developmental stability. Cold Spring Harbor Symposium Quant. Biology 22:318-326.

Thomas, R. and R. Poulin. 1997. Using randomization techniques to analyse fluctuating asymmetry. Animal Behavior 54:1027-1029.

Tracy, M., D. C. Freeman, J. M. Emlen, J. H. Graham, and R. A. Hough. 1995. Developmental instability as a biomonitor of environmental stress. Pages 313-337 In Biomonitors and Biomarkers as Indicators of Environmental Change. Butterworth, F. M. et al. (eds.) Plenum Press, New York.

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Valentine, D. W. and M. Soulé. 1973. Effect of p,p'-DDT on developmental stability of pectoral fin rays in the grunion, Leuresthes tenuis. Fishery Bulletin 71:921-926.

Valentine, D. W. and M. E. Soulé, and P. Samallow. 1973. Asymmetry analysis in fishes: A possible statistical indicator of environmental stress. Fishery Bulletin 71:357-370.

Van Valen, L. 1962. A study of fluctuating asymmetry. Evolution 16:125-142.

Vrijenhoek, R. C. and S. Lerman. 1982. Heterozygosity and developmental stability under sexual and asexual breeding systems. Evolution 36:768-776.

Watson, P. J. and r. Thornhill. 1994. Fluctuating asymmetry and sexual selection. Trends in Ecology and Evolution 9:21-25.

Wiener, J. G. and P. J. Rago. 1987. A test of fluctuating asymmetry in bluegills (*Lepomis macrochirus* Rafinesque) as a measure of pH-related stress. Environmental Pollution 44:27-36.

Zakharov, V. M. and V. V. Zyuganov. 1980. Assessment of asymmetry of bilateral characters as a population characteristic. Ékologiya 1:10-16.

Zakharov, V. M. and J. H. Graham. 1992a. Developmental stability in natural populatons. Introduction. Acta Zoologica Fennica 191:4-5.

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Zakharov, V. M. and J. H. Graham. (eds.) 1992b. Developmental Stability in Natural Populations. Acta Zoologica Fennica No. 191.

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CHAPTER 2

ASSESSMENT OF FLUCTUATING ASYMMETRY THROUGH MAXIMUM LIKELIHOOD ESTIMATES BASED ON ALTERNATIVE DEVELOPMENTAL MODELS

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ABSTRACT

Fluctuating asymmetry, random variation of a meristic or morphometric characteristic from one side of an organism to the other, has been proposed as a population index of developmental stress resulting from pollution, extreme genetic homozygosity or heterozygosity, or environmental conditions. However, in many instances, the usual measures of fluctuating asymmetry in meristic characters have been inconsistent in distinguishing among different populations exposed to varying levels of contaminants or with different amounts of genetic variation. An alternate measure of fluctuating asymmetry, $\hat{\sigma}_a$, is described which overcomes some of the biases and other statistical problems of fluctuating asymmetry measures based on differences in bilateral counts. Maximum likelihood estimates are determined by a numerical search algorithm.

The new index of asymmetry is far less sensitive to bias from variation in the mean character value than previously used indices. Likelihood ratio tests for the new index are shown to exhibit nominal levels of α-error, and yet are as or more powerful than tests for the alternative measures.

Application of the new index to bilateral character data for lake chub (Coesius plumbeus) demonstrated the increased power at distinguishing differences in fluctuating asymmetry.

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INTRODUCTION

In a description of the different types of biological asymmetry, Van Valen (1962) described fluctuating asymmetry as a random difference in the magnitude or count of a bilateral characteristic between one side of an organism and another. In fluctuating asymmetry, the difference in value of the character between the two sides has a normal distribution centered on zero. Other types of asymmetry also exist, such as directional asymmetry and antisymmetry, but these types have different statistical characteristics (Palmer 1994).

In the 36 years since Van Valen's paper was published, fluctuating asymmetry has been examined in a great diversity of organisms as a measure of stress during the developmental process. Stress, from a variety of sources such as environmental conditions, pollutants, or genetic heterozygosity, is believed to cause minor disturbances of development that result in asymmetrical bilateral characters that, under ideal circumstances, would be symmetric.

Swain (1987) described the conceptual developmental model which forms the basis for fluctuating asymmetry of meristic characters. The character state (realized number of countable elements of a meristic character) is determined from a normal distribution called the liability. The mean of the liability, μ, is a characteristic of the population to which the organism belongs. As each side of the organism develops, minor disturbances result in slight independent

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deviations from μ. These deviations have a variance of σ_a^2. The actualized value along the liability, i. e. the mean plus the disturbance, is translated into discrete characters depending on specific threshold values in the distribution of liability. In essence, the actualized value is rounded to an integer. For example, with $\mu = 10.3$ and a deviation due to developmental disturbance of +0.3, and threshold values half-way between integers, the realized character value would be 11. Developmental deviations between -0.8 and +0.2 would result in character values of 10.

The basis for most statistical analysis of fluctuating asymmetry has been that the difference between character values on right and left sides would vary according to a normal distribution with a mean of zero (Van Valen 1962). The variance of the difference is the most commonly used measure of fluctuating asymmetry and has been shown for morphometric characters to be preferred over many alternative measures (Palmer and Strobeck 1992).

Meristic characters, due to the fact that they are determined by thresholds along the underlying normal liability, have an inherent difficulty of measurement when the actual amount of asymmetry variation is small. If μ is an integer value (a), any deviation whose absolute magnitude is less than 0.5 results in a realization of a. However, if $\mu = a.5$, then even a very small deviation, e.g. +0.01, results in an observed character value of a+1, while a deviation of -0.01 results in a. Thus the observed

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character values are more sensitive to small asymmetry variations when μ is halfway between integer values and less sensitive when μ is at an integer value.

Asymmetry statistics based on the difference in right and left side counts are more sensitive to the value of μ when deviations in the character values are low, i.e. typical differences are between -1 and +1 (Swain 1987, Palmer 1994). This leads to great difficulty in distinguishing asymmetry differences among populations or in using fluctuating asymmetry as a measure of developmental stress, unless the stress is severe enough to generate larger asymmetry values.

In this paper I propose using estimates of the asymmetry standard deviation (σ_a) as the measure of fluctuating asymmetry. In the course of this effort, a more general model of development and fluctuating asymmetry with one additional parameter was developed. This model is fit to the actual right and left character values rather than to their differences, and thus incorporates more of the information from the data than do the common measures of fluctuating asymmetry. In addition to the model fitting, the influence of μ on the estimate of asymmetry, power of hypothesis tests, sensitivity of the asymmetry measures to counting errors, and examples of application to actual data are provided.

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METHODS

DEVELOPMENTAL MODELS

The character state of bilateral meristic characters can be described as a sum of continuous random variables rounded to the nearest integer:

$Y_{ij} = \mathrm{Round}(X_{ij} = \mu + \delta_i + e_{ij})$

where Y_{ij} = character state on side j of organism i

X_{ij} = realized value of liability on side j of organism i

μ = mean of liability for the population

δ_i = deviation from mean liability for organism i

$\sim \mathrm{Normal}(0,\sigma_L^2)$

e_{ij} = deviation due to developmental instability on side j of organism i

$\sim \mathrm{Normal}\ (0,\sigma_a^2)$

This model can also be described as the rounded value of the sum of the individual liability plus the deviation due to developmental disruption:

$Y_{ij} = \mathrm{Round}(X_{ij} = L_i + e_{ij})$

where $L_i = \mu + \delta_i$

$\sim N(\mu,\sigma_L^2)$

This mathematical model thus accounts separately for variation of the character value among organisms with parameters μ and σ_L and for developmental disruptions that result in fluctuating asymmetry using σ_a. The conceptual developmental model described by Swain (1987) is a special case of this model in which $\sigma_L \equiv 0$.

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The joint probability density function for X_{ij} on the right and left sides of the organism is:

$$f(X_{ir},X_{il}) = f(X_{ir}|L_i)\bullet f(X_{il}|L_i)\bullet f(L_i)$$

$$f(x_{ir},x_{il}) = \int_{L_i=-\infty}^{\infty} \frac{1}{2\pi\sigma_a^2}\exp\left[\frac{(x_{ir}-L_i)^2+(x_{il}-L_i)^2}{-2\sigma_a^2}\right]\frac{1}{\sqrt{2\pi}\sigma_L}\exp\frac{(L_i-\mu)^2}{-2\sigma_L^2}dL_i$$

where X_{ir} and X_{il} are iid $N(L_i,\sigma_a^2)$ and L_i is $N(\mu,\sigma_L^2)$

Since the realized character values (Y_{ij}) must be integers, the development process is assumed to have threshold levels which translate the continuous distribution of the liability into integer-valued observations. The probability of an observation $Y_{ir} = a$ and $Y_{il} = b$ (a and b are integers) is then found from

$$Pr(Y_{ir}=a, Y_{il}=b) = \int_{x_{ir}=a-.5}^{a+.5}\int_{x_{il}=b-.5}^{b+.5} f(x_{ir},x_{il})\,dx_{ir}dx_{il}$$

For the reduced two-parameter model described by Swain (1987), the joint probability density of the liability can be simplified to:

$$f(X_{ir},X_{il}) = f(X_{ir})\bullet f(X_{il})$$

where X_{ir} and X_{il} are distributed iid $N(\mu,\sigma_a^2)$

$$f(x_{ir},x_{il}) = \frac{1}{2\pi\sigma_a^2}\exp-\left[\frac{(x_{ir}-\mu)^2+(x_{il}-\mu)^2}{2\sigma_a^2}\right]$$

The probability of any particular observation (a,b) is again found by integrating the density function between $(a-.5) < x_{ir} < (a+.5)$, $(b-.5) < x_{il} < (b+.5)$.

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PARAMETER ESTIMATION

In order to evaluate these models of character development, I constructed a computer algorithm to estimate the model parameters (μ, σ_L, σ_a) for simulated or actual data using maximum likelihood methods (Hilborn and Mangel 1997). The basic method used to find the set of parameter estimates that maximized the likelihood ($\hat{\mu}, \hat{\sigma}_L, \hat{\sigma}_a$) was a gradient search, a general method for solving multidimensional maximization problems (Hillier and Lieberman 1980). Following usual practice (Hilborn and Mangel 1997), the search actually minimized the negative $\log_e$ of the likelihood (NLL).

For the three-parameter (full) model:

$$NLL_3(\mu, \sigma_L, \sigma_a | y_r, y_l) = -\sum_{i=1}^{n} \log_e \int_{L_i=-\infty}^{\infty} \exp -[\frac{(y_{ir}-L_i)^2+(y_{il}-L_i)^2}{2\sigma_a^2} + \frac{(L_i-\mu)^2}{2\sigma_L^2}] dL_i - \sum_{i=1}^{n} \log_e(2\pi\sigma_a^2 + \sqrt{2\pi}\sigma_L)$$

and for the two-parameter (reduced) model:

$$NLL_2(\mu, \sigma_a | y_r, y_l) = -\sum_{i=1}^{n} -[\frac{(y_{ir}-\mu)^2+(y_{il}-\mu)^2}{2\sigma_a^2}] - \log_e(2\pi\sigma_a^2)$$

The method begins by examining the partial derivatives of NLL with respect to each of the parameters at an initial trial estimate. The trial estimate is then adjusted in small increments in the direction of the vector sum of the partial derivatives (gradient). As the trial estimates

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change along the gradient, NLL will initially decrease but at some point it may begin to increase. The current parameter estimates are then used as a new trial estimate to recalculate the gradient and the adjustments begin again in the direction of the new gradient. The process is repeated until the gradient is sufficiently close to zero or until no further decrease in NLL is possible.

The integrals in the probability equations were solved by numerical approximation. The definite integrals were approximated by evaluating the density at points spread evenly over the 1 x 1 cell centered on (a,b). The number of points used to evaluate the integral ranged from 25 for estimated $\sigma_a > 0.5$ to 625 for $\sigma_a < 0.1$. The increased number of points was necessary to accurately estimate the mean cell density when the density changed abruptly within a cell at small values of σ_a.

Initial estimates for the model parameters (μ,σ_L,σ_a) were taken from a one-way ANOVA using the individuals as blocks. The grand mean was the initial estimate of μ. The $\sqrt{\sigma^2_{Blocks}}$ and $\sqrt{\sigma^2_{Error}}$ served as initial estimates for σ_L and σ_a for the full model, while $\sqrt{MS_{Total}}$ was the initial estimate of σ_a for for the reduced model.

The search algorithms were tested for proper convergence to true parameter values using simulated data prior to using them on actual data. Verification of the algorithms was done by generating a data set from a particular set of parameter values (μ,σ_L,σ_a). These data

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sets contained values, even fractional values, for all cells (y_r, y_l) that had an average probability within the cell over 0.001. To demonstrate unbiased estimation the search algorithm for the correct model, i.e. full or reduced, had to be able to converge closely to the true parameter values, with perhaps less close convergence for the incorrect model. The search algorithm and all other calculations were implemented in APL (APL2000) under the Windows95 operating system (Microsoft Corporation). The programs written to conduct the analyses are available from the author upon request.

ASYMMETRY INDICES BASED ON DIFFERENCES

Asymmetry parameter estimates for the two models were compared to previously used asymmetry indices based on the within-individual difference in character values:

$$A_i \quad = Y_{ir} - Y_{il}$$

The sample variance of A is the measure of fluctuating asymmetry recommended by Palmer and Strobeck (1986). I used the sample standard deviation, s_A (Table 1) in order to compare the estimator directly with σ_a.

Other asymmetry indices used were: $\hat{p}$, the proportion of individuals that are asymmetric; $|\overline{A}|$, mean of the absolute value of A; and $\hat{q}$, parameter for a double geometric distribution for A (Table 2-1). The double geometric distribution was examined because it might provide better estimates in situations where the distribution of A is

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leptokurtotic, as has sometimes been the case in empirical studies (Jagoe and Haines 1985, Felley 1980). All of these parameters have closed form estimates based on the data. Proof that $\hat{q}$ is an unbiased maximum likelihood estimate of q is given in the appendix.

EVALUATION OF INDICES AND TESTS

Once the algorithms to estimate the model-based asymmetry parameters were shown to correctly converge, the stability of the each of the indices as μ varied between integer and half-integer values and σ_L and σ_a are small was evaluated. Precision of the asymmetry indices was determined by repeated sampling from distributions with known parameter values. Samples of a fixed size (50) were taken and the mean, standard deviation, and CV for each of the asymmetry indices were determined. Normality of parameter estimates was evaluated by examining skewness (g_1) and kurtosis (g_2) (Palmer 1994).

Model-based hypothesis tests for differences in σ_a between two samples were constructed from likelihood ratio tests. Consistent with use of the negative $\log_e$ of the likelihood, the tests were calculated from the $\log_e$ of the likelihood ratio (LLR). (2 x LLR) has a χ^2 distribution with 1 degree of freedom.

$$\begin{aligned} LLR_3 = {} & NLL_3(\mu_1, \sigma_{L1}, \sigma_a | sample1) + NLL_3(\mu_2, \sigma_{L2}, \sigma_a | sample2) \\ & - NLL_3(\mu_1, \sigma_{L1}, \sigma_{a1} | sample1) - NLL_3(\mu_2, \sigma_{L2}, \sigma_{a2} | sample2) \end{aligned}$$

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$$\begin{aligned} LLR_2 = {} & NLL_2(\mu_1, \sigma_a | sample1) + NLL_2(\mu_2, \sigma_a | sample2) \\ & - NLL_2(\mu_1, \sigma_{a1} | sample1) - NLL_2(\mu_2, \sigma_{a2} | sample\ 2) \end{aligned}$$

Likelihood ratio tests are typically the most powerful tests possible when the data are of the appropriate distributional form (Mood et al. 1974). Tests used for the difference-based indices were F-test for s_A^2, Fisher's exact test for $\hat{p}$, t-test for $|\bar{A}|$, and likelihood ratio test for $\hat{q}$ (Table 2-1).

To evaluate power of the hypothesis tests for $H_0{:}\sigma_{a1} = \sigma_{a2}$, 200 pairs of bootstrap samples of size 50, approximate typical sample size for many published asymmetry studies, were drawn from simulated Model 2 data with $\mu = 10$ and $\sigma_L = 0.5$. A base asymmetry level was set at $\sigma_a = 0.5$, and various departures above and below that level ranging from 0.125 to 0.875. Samples of 50 were drawn randomly from the base and departure distributions, and the asymmetry measures were tested for significant differences at the $\alpha = 0.05$ level. Type I (α) error (probability of rejecting a true H_0) was examined by testing two independent samples from the base asymmetry distribution. Power, the converse of β error (probability of accepting a false H_0), was estimated from the proportion of significant tests when each of the

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Table 2-1. Asymmetry indices and statistical tests based on differences in right and left side counts.

Asymmetry		Statistical tests	
Index	Calculation	2-Sample	Multi-sample
s_A	$\sqrt{}(\sum A_i^2/n)$	F-test	F_{max}
$\hat{q}$	$\sum\|A_i\|/(n+\sum\|A_i\|)$	Likelihood Ratio	Likelihood Ratio
$\|\bar{A}\|$	$\sum\|A\|/n$	t-test	Kruskal-Wallis
$\hat{p}$	$(\sum A')/n$	Fisher exact	G-test

where A_i = observed value of $y_{ir}-y_{il}$

A_i' = minimum ($|A_i|$, 1)

n = number of individuals in the sample

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departure distributions was tested against the base distribution.

Sensitivity of each of the indices to errors in counting meristic characters was determined by adding counting errors to simulated meristic data. The error distribution was assumed to be double geometric, in which the parameter q is the expected fraction of observations that have errors in the count. Errors could be either positive or negative.

Empirical data on asymmetry of meristic characters was obtained from a previously reported study of fluctuating asymmetry in fish in relation to pH (Jagoe and Haines 1985). The results for lake chub (*Coesius plumbeus*) collected from two acid stressed sites in New York's Adirondack region (North Lake and South Lake) and from a higher pH site in Maine (Penobscot River) are presented to illustrate the new indices and tests. Multi-sample tests were used to test differences in fluctuating asymmetry among the three locations. Differences in σ_a were tested by extending the likelihood ratio test to multiple samples. Individual estimates of μ and σ_L (for the full model) were fit to each population while a single σ_a was fit to all sites. The total NLL for all the data using a single estimate of σ_a was compared to the total NLL using individual estimates of σ_a for each site. Other multi-sample tests used for comparison were Kruskal-Wallis test for $|\bar{A}|$, F_{max} test for s_A^2, and G-test for $\hat{p}$ (Sokal and Rohlf 1993).

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RESULTS

VALIDATION OF ESTIMATION ALGORITHMS

For data generated by the full model, with $\sigma_L = 0.25$, only the estimate of σ_a based on the full model (labelled sigaF in the figures) gave unbiased estimates over the range $0.025 \leq \sigma_a \leq 2.5$ (Figure 2-1). $|\bar{A}|$ (meanA) was the next most consistent estimator, showing only a slight positive bias. s_A (stdA) and σ_a from the reduced model (sigaR) were severely biased high at low values of σ_a, but tended to converge to σ_a at higher values, although s_A still had some bias at $\sigma_a = 2.5$. Estimates of p and q matched σ_a at low values of σ_a but became negatively biased as σ_a increased.

For data generated by the reduced model ($\sigma_L = 0$), only the σ_a estimate based on this model (sigaR) was unbiased over the entire range examined, $0.25 \leq \sigma_a \leq 2.5$ (Figure 2-2) The full model estimate (sigaF) was slightly biased low at true $\sigma_a = 0.25$ and slightly high at $\sigma_a = 0.50$. At larger values of σ_a, sigaF was identical to sigaR.

All of the indices based on A showed substantial, and variable biases. s_A (stdA) was biased high over the entire range of σ_a, while $\hat{q}$ and $\hat{p}$ showed a variable degree of negative bias. $|\bar{A}|$ (meanA) was negatively biased at the lowest σ_a but slightly positively biased at higher values.

In general, the NLL values calculated for the full and reduced model parameters could successfully distinguish which of the models had actually generated the data only

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Figure 2-1 Asymmetry index values as a function of true σ_a. Values estimated from full model distribution with $\mu = 10.0$, $\sigma_L = 0.25$.

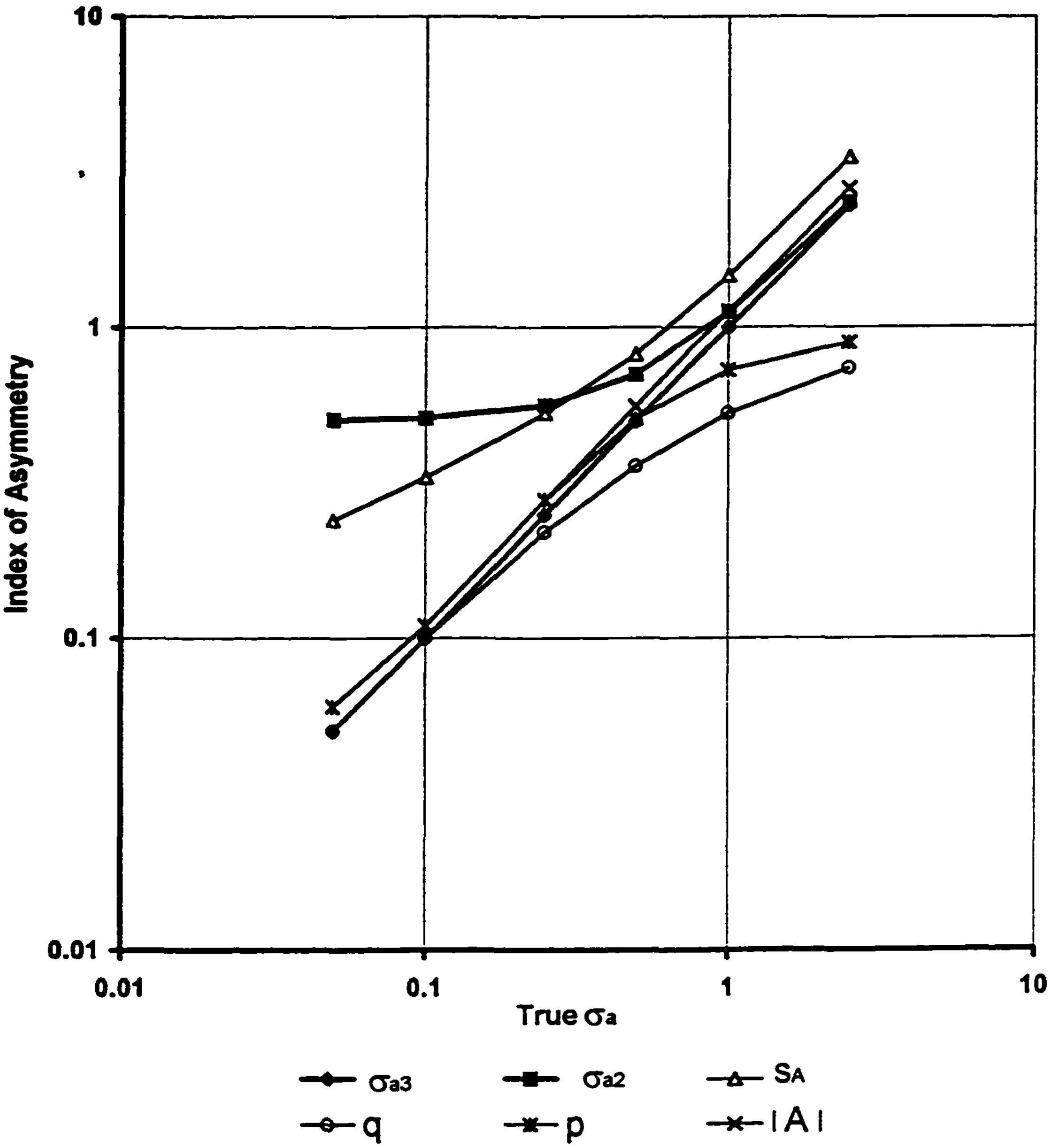

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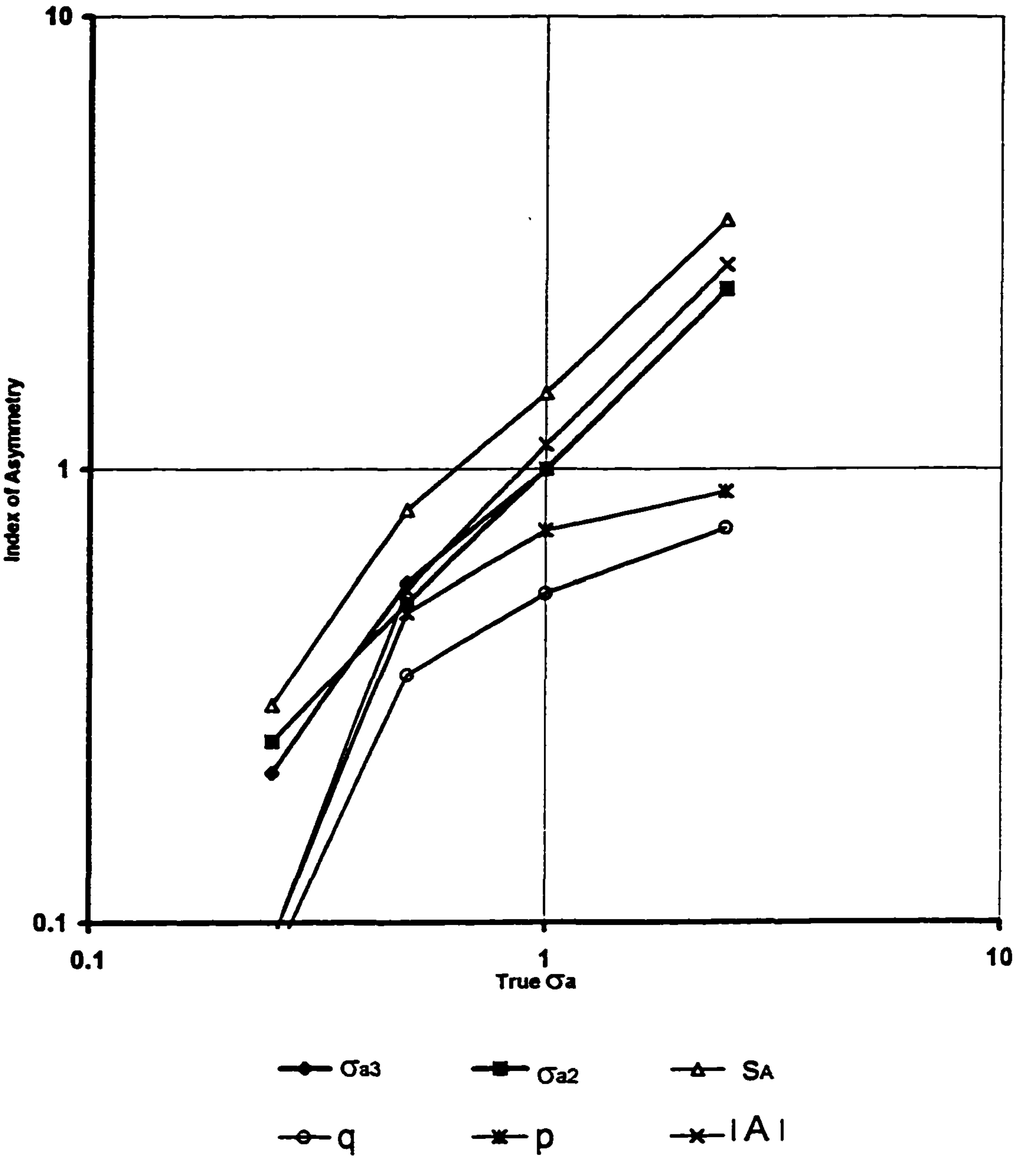
Figure 2-2 Asymmetry index values as a function of true σ_a. Values estimated from reduced model distribution with μ =10.0, σ_L = 0.



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when the full model was true underlying model. With σ_L = 0.25, the NLL for the full model was always less than for the reduced model, although NLL values converged as σ_a increased and the relative contribution of σ_L to the total variability decreased. For the reduced model ($\sigma_L = 0$), the NLL values were identical for $\sigma_a > 1.0$, but at $\sigma_a = 0.5$ the NLL for the full model was slightly lower. At the lowest σ_a (= 0.25), the algorithm for the full model could not properly calculate the NLL and produced a negative estimate, although an estimate of σ_a was still produced. This calculation problem occurred only in situations where there was so little variation in the data that the standard deviation of the character value was less than 0.22.

The two model-based estimates of σ_a proved to be much less sensitive to the value of μ than are the asymmetry statistics based on A (Figure 2-3). When μ was varied from 10.0 to 10.5, with $\sigma_L = 0.25$ and true $\sigma_a = 0.1$, sigaR and sigaF were constant except at $\mu_L = 10.5$ when they decreased slightly. Thus sigaF exhibited almost no bias, while sigaR exhibited a nearly constant level of bias. In contrast, s_A (stdA), $\hat{q}$, $\hat{p}$, and $|\overline{A}|$ (meanA) all tended to increase as μ departed further from an integer value. $\hat{q}$, $\hat{p}$, and $|\overline{A}|$ were negatively biased when the shift from an integer value of μ was ≤ 0.2 and positively biased at higher values. All estimators exhibited identical behavior as μ decreased from 10.0 to 9.5.

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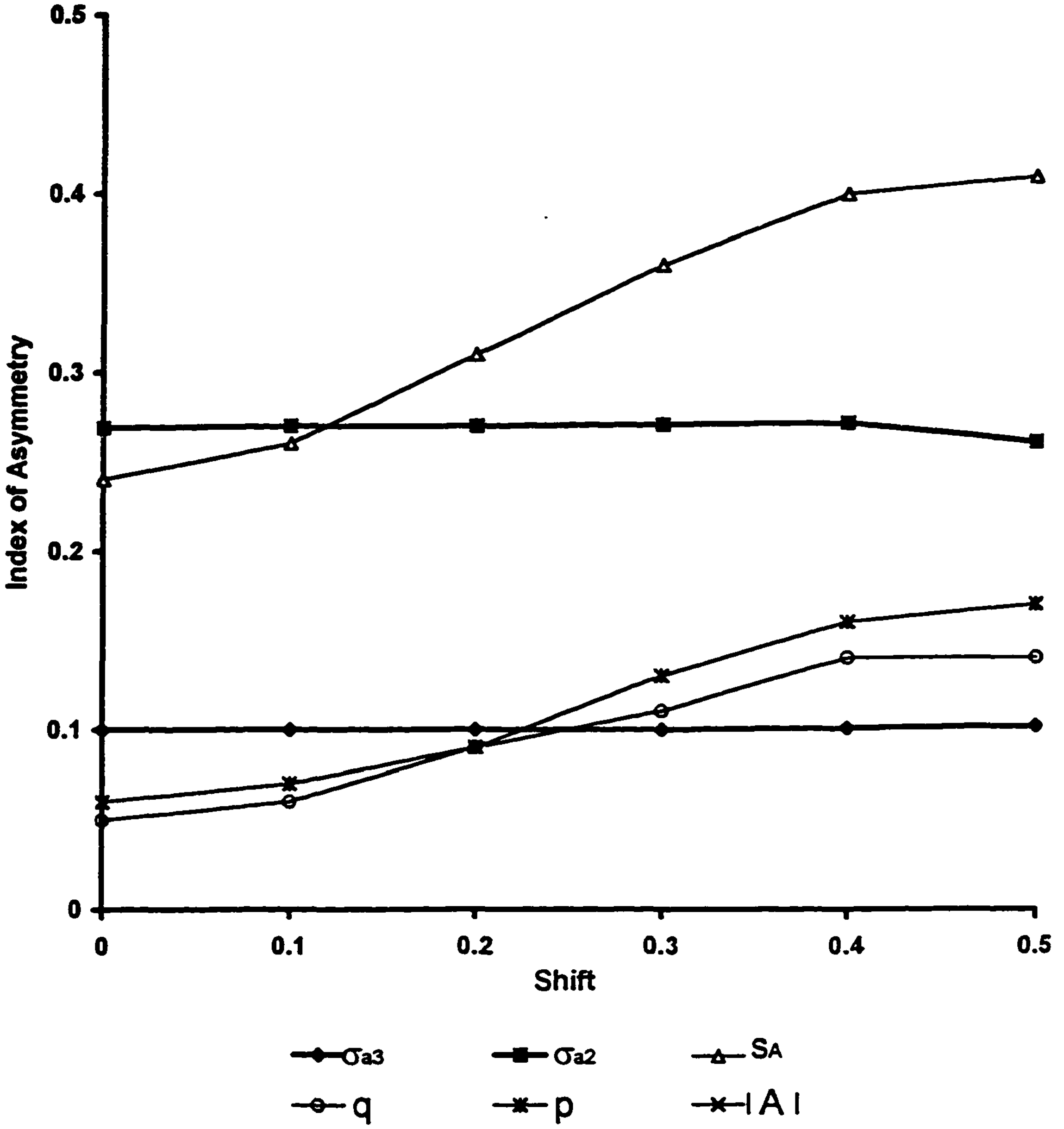
Figure 2-3 Asymmetry index values as a function of shift from integer value of μ. Values estimated from full Model 2 distribution with $9.5 \le \mu \le 10.0$, $\sigma_L = 0.25$.



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The maximum severity of the bias was examined by setting μ at 10.5 and varying σ_a for the reduced model (Figure 2-4) and σ_L for the full model (Figure 2-5). For the reduced model, sigaR was constant at 0.2 for true $\sigma_a < 0.2$, and equal to σ_a for $\sigma_a \geq 0.2$. All of the other estimators, including sigaF, overestimated σ_a at low values of σ_a, but sigaF, $\hat{p}$, and $|\bar{A}|$ converged considerably at $\sigma_a = 0.5$.

For the full model ($\mu_L = 10.5$, $\sigma_a = 0.1$), all estimators were positively biased at low values of σ_L, i.e. as the model approached a reduced model situation. sigaF was the least biased and converged to true σ_a at $\sigma_L = 0.25$. $|\bar{A}|$, $\hat{q}$, and $\hat{p}$ all had more substantial bias but converged to σ_a at $\sigma_L = 0.5$. s_A (sA) showed decreasing bias but never converged, while sigaR showed increasing bias as σ_L increased.

EVALUATION OF MODELS

Prior to fitting the models to the lake chub data, the data were checked to see whether they fit the proper pattern for fluctuating asymmetry (Palmer 1994). Examination of the A values indicated one instance in which the mean value of A was significantly different from 0 using a t-test (Table 2-2). However, Jagoe and Haines (1985) found that all of these characters (pooled over all three locations) were significantly non-normal (Kolmogorov-Smirnov statistic) and gill rakers, pelvic fin rays, radii on the A scale, and

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Figure 2-4 Asymmetry index values as a function of true σ_a. Values estimated from full Model 1 distribution with μ =10.5, σ_L = 0.

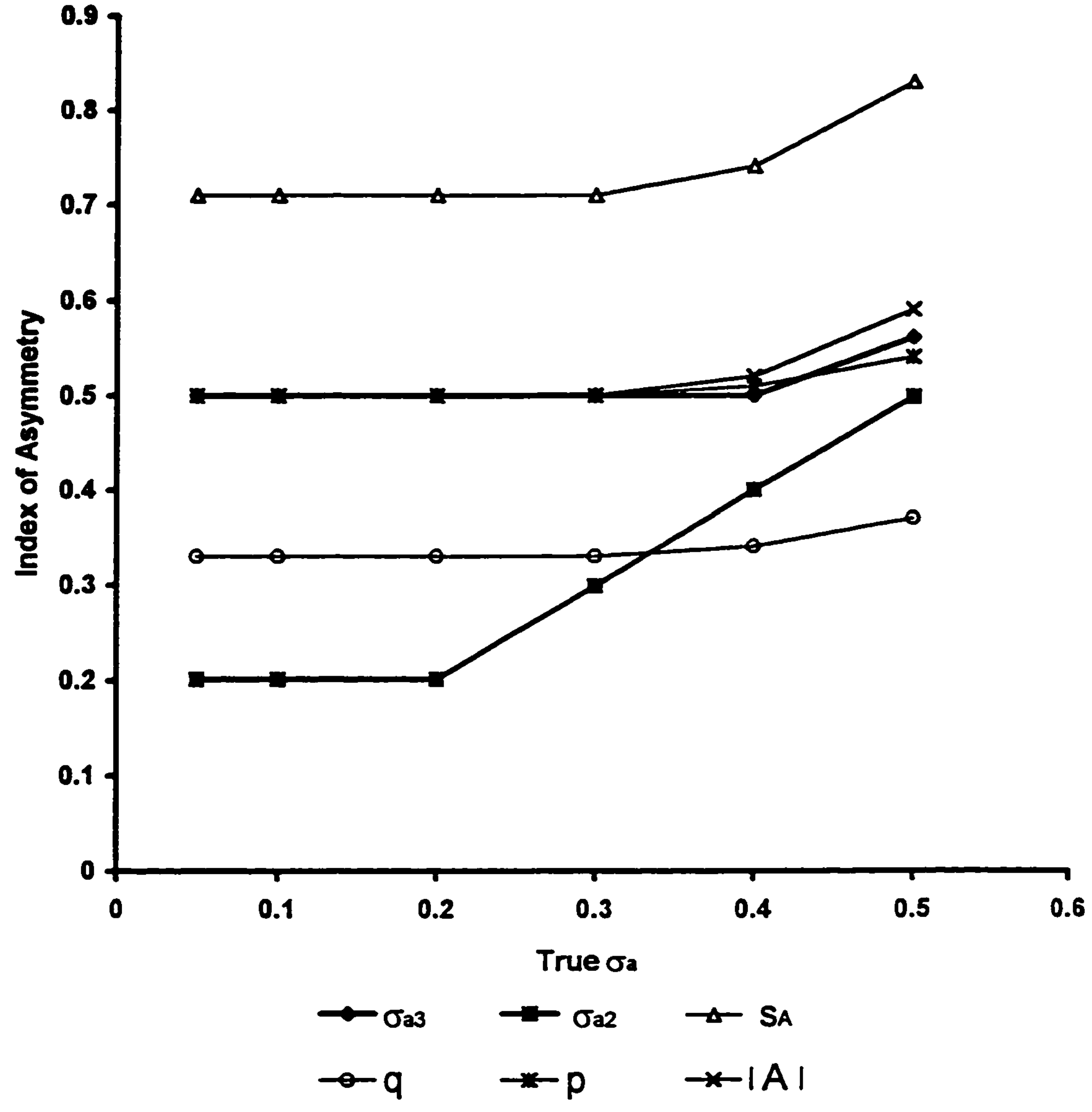

Figure 2-5 Asymmetry index values as a function of σ_L. Values estimated from full Model 2 distribution with μ =10.5, σ_a = 0.10.

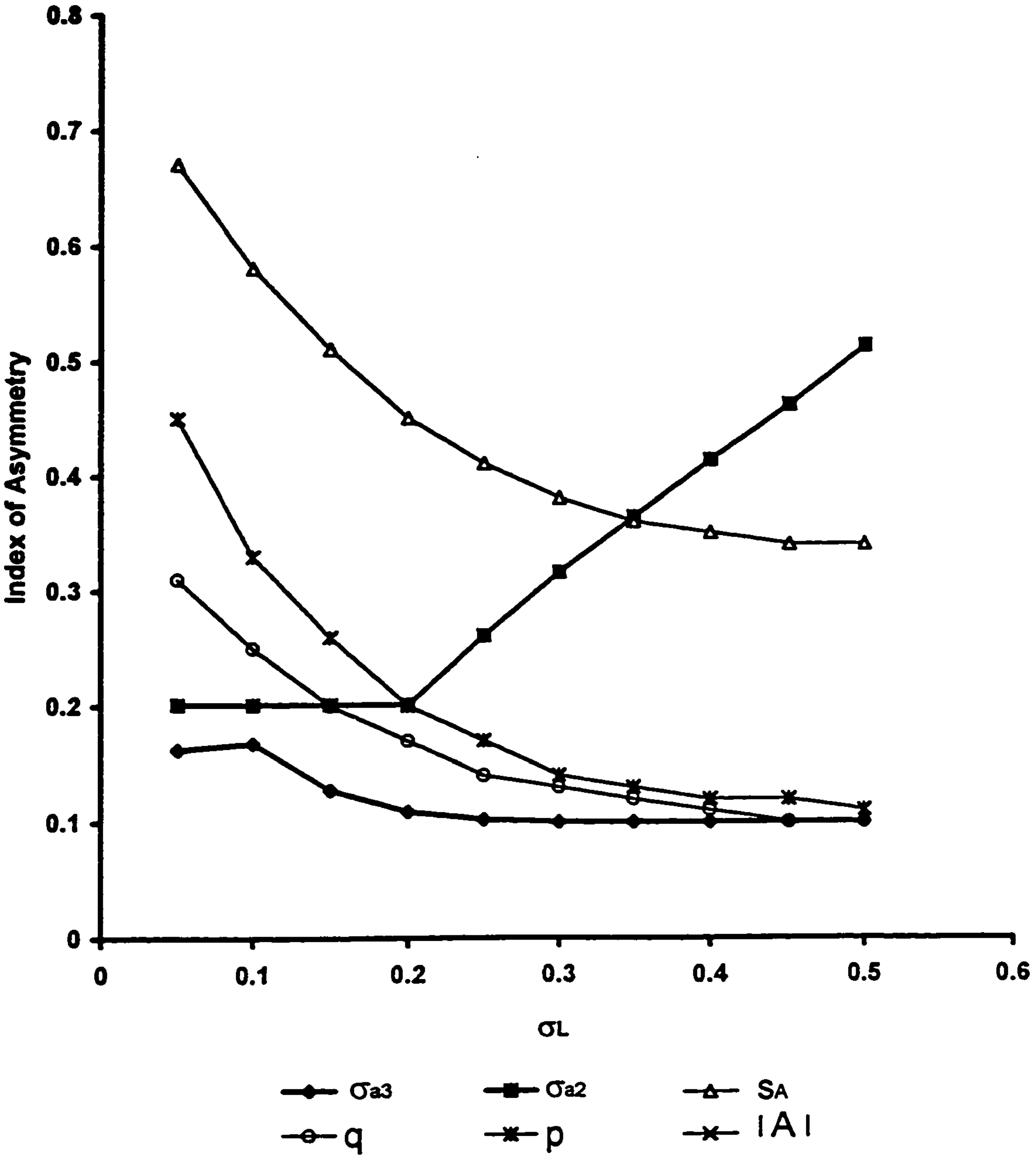

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Table 2-2 Statistics of meristic characters for three populations of lake chub.

		Character		Tests for normality of A		
Character	n	Mean	Std	Direction	Skewness	Kurtosis
Lateral line scales	110	64.70	3.75	ns	ns	ns
Scales above lateral line	109	12.12	0.90	ns	ns	<0.01
Scales below lateral line	109	8.93	0.92	<0.05	ns	<0.01
Pectoral fin rays	46	16.80	0.88	ns	ns	<0.01
Pelvic fin rays	110	8.11	0.38	ns	<0.05	<0.01
Radii on A scale	109	9.34	4.86	ns	ns	<0.05
Radii on B scale	110	13.11	4.65	ns	ns	ns
Gill Rakers	105	10.93	1.57	ns	ns	<0.01

ns indicates $p>0.05$

scales below the lateral line were all significantly leptokurtotic. This analysis revealed one instance of significant ($\alpha = 0.05$) skewness and significant leptokurtosis for all but 2 characters.

Fitting of the two models to various meristic characters of lake chub indicated that the full model typically fit better than the reduced model, as indicated by a lower NLL (Table 2-3). There was one instance, pelvic fin rays for fish from the Penobscot River, in which NLL for the full model was negative, indicating difficulty in fitting. The character mean for this group was an integer value (8.00) and the character standard deviation was 0.17, a very low amount of variation. Of the 37 individuals in the sample, 35 were (8,8), 1 was (8,7) and the remaining one was (8,9). Although the search algorithm for the full model produced an estimate of σ_a (0.164) for this group, comparison of other estimates from the full model and $|\bar{A}|$ suggests that this value of $\hat{\sigma}_a$ is too high. The reduced model estimate of σ_a is also suspect since this is the only case in which $\hat{\sigma}_a$ exceeded the character standard deviation. Thus the empirical results confirm the results of the generated data in that the estimation algorithm breaks down when the character standard deviation is less than approximately 0.2.

POWER OF TESTS

Data for this analysis were generated from the full

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Table 2-3 Model fits and asymmetry parameter estimates for lake chub meristic characters. n = sample size; x̄ = sample character mean; s = sample character standard deviation; NLL = negative log likelihood; $\hat{\sigma}_L$ = estimated standard deviation of liability; $\hat{\sigma}_a$ = estimated asymmetry standard deviation.

	Character			Reduced Model		Full Model			Statistics for A				
Lateral line scales													
Location	n	x̄	s	NLL	$\hat{\sigma}_a$	NLL	$\hat{\sigma}_L$	$\hat{\sigma}_a$	$\bar{A}$	s_A	$\hat{q}$	$\hat{p}$	$\lvert\bar{A}\rvert$
North Lake	24	65.4	3.3	124.2	3.203	119.6	2.42	2.109	¯0.63	3.01	0.71	0.96	2.46
South Lake	49	66.2	3.7	267.6	3.696	246.7	3.24	1.805	¯0.08	2.59	0.68	0.82	2.12
Penobscot	37	62.3	2.8	182.1	2.824	170.7	2.33	1.580	0.57	2.27	0.62	0.65	1.65
Scales Above Lateral Line													
Location	n	x̄	s	NLL	$\hat{\sigma}_a$	NLL	$\hat{\sigma}_L$	$\hat{\sigma}_a$	$\bar{A}$	s_A	$\hat{q}$	$\hat{p}$	$\lvert\bar{A}\rvert$
North Lake	23	11.7	1.1	68.7	1.045	66.9	0.67	0.806	¯0.13	1.20	0.45	0.65	0.83
South Lake	49	12.1	0.8	113.5	0.717	105.4	0.56	0.445	0.06	0.74	0.32	0.43	0.47
Penobscot	37	12.4	0.8	89.0	0.754	77.1	0.67	0.341	¯0.03	0.64	0.29	0.41	0.41

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Table 2-3 Continued

Scales Below Lateral Line

Location	n	$\bar{x}$	s	NLL	$\hat{\sigma}_a$	NLL	$\hat{\sigma}_L$	$\hat{\sigma}_a$	$\bar{A}$	s_A	$\hat{q}$	$\hat{p}$	$\lvert\bar{A}\rvert$
North Lake	23	9.6	0.9	57.58	0.796	51.7	0.67	0.429	¯0.26	0.72	0.30	0.39	0.43
South Lake	49	8.6	0.8	110.74	0.693	107.4	0.44	0.534	0.39	0.85	0.34	0.43	0.51
Penobscot	37	8.9	0.9	100.47	0.896	84.7	0.82	0.356	0.11	0.66	0.30	0.43	0.43

Pectoral Fin Rays

Location	n	$\bar{x}$	s	NLL	$\hat{\sigma}_a$	NLL	$\hat{\sigma}_L$	$\hat{\sigma}_a$	$\bar{A}$	s_A	$\hat{q}$	$\hat{p}$	$\lvert\bar{A}\rvert$
North Lake	9	17.0	1.0	25.54	0.956	24.5	0.67	0.686	¯0.67	1.05	0.47	0.78	0.89
Penobscot	37	16.8	0.8	91.73	0.788	76.0	0.73	0.320	0.00	0.57	0.21	0.24	0.27

Pelvic Fin Rays

Location	n	$\bar{x}$	s	NLL	$\hat{\sigma}_a$	NLL	$\hat{\sigma}_L$	$\hat{\sigma}_a$	$\bar{A}$	s_A	$\hat{q}$	$\hat{p}$	$\lvert\bar{A}\rvert$
North Lake	24	8.1	0.4	29.57	0.371	25.9	0.34	0.168	¯0.17	0.41	0.14	0.17	0.17
South Lake	49	8.2	0.4	58.90	0.349	44.0	0.35	0.080	0.04	0.29	0.08	0.08	0.08
Penobscot	37	8.0	0.2	10.58	0.227	¯4.6	0.02	0.164	0.00	0.23	0.05	0.05	0.05

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Table 2-3 Continued

Radii on A Scale

Location	n	$\bar{x}$	s	NLL	$\hat{\sigma}_a$	NLL	$\hat{\sigma}_L$	$\hat{\sigma}_a$	$\bar{A}$	s_A	$\hat{q}$	$\hat{p}$	$\lvert\bar{A}\rvert$
North Lake	24	15.2	3.8	132.13	3.787	129.5	2.52	2.831	¯0.29	4.02	0.75	0.83	2.96
South Lake	49	10.0	3.0	247.93	3.022	243.2	1.98	2.292	¯0.61	3.27	0.71	0.86	2.45
Penobscot	36	4.5	1.6	136.32	1.584	134.5	0.91	1.301	0.00	1.89	0.58	0.75	1.39

Radii on B Scale

Location	n	$\bar{x}$	s	NLL	$\hat{\sigma}_a$	NLL	$\hat{\sigma}_L$	$\hat{\sigma}_a$	$\bar{A}$	s_A	$\hat{q}$	$\hat{p}$	$\lvert\bar{A}\rvert$
North Lake	24	17.3	4.0	134.67	3.994	128.5	3.19	2.416	0.08	3.43	0.74	0.88	2.83
South Lake	49	14.5	3.3	256.57	3.303	248.8	2.39	2.271	¯0.35	3.24	0.70	0.82	2.31
Penobscot	37	8.6	2.1	158.74	2.050	153.7	1.44	1.456	0.49	2.09	0.62	0.81	1.62

Gill Rakers

Location	n	$\bar{x}$	s	NLL	$\hat{\sigma}_a$	NLL	$\hat{\sigma}_L$	$\hat{\sigma}_a$	$\bar{A}$	s_A	$\hat{q}$	$\hat{p}$	$\lvert\bar{A}\rvert$
North Lake	23	11.7	1.1	69.84	1.066	62.2	0.92	0.542	¯0.04	0.86	0.32	0.35	0.48
South Lake	46	11.6	1.3	153.25	1.249	147.9	0.87	0.904	0.00	1.34	0.49	0.65	0.96
Penobscot	36	9.6	1.3	122.39	1.249	110.0	1.11	0.667	¯0.03	1.01	0.35	0.36	0.53

model since this it clearly fit empirical data better than the reduced model. Power curves for two-sample tests indicated the superiority of the likelihood ratio test for σ_a (Figure 2-6). Ideally, the proportion of significant results is equal to the nominal α level when two samples are drawn from the same population, then increases rapidly as the true parameter of the two samples diverge. The full model likelihood ratio test (LLR_3) was the only test that approximated the nominal α level (0.05) when both samples had a true $\sigma_a = 0.5$ and also had the highest power for detecting real differences in σ_a. The t-test for differences in $|\bar{A}|$ (t), and likelihood ratio test for q (LRq) had an α error rate of near 0, and low power over all the levels of σ_{a2} examined. The reduced model likelihood ratio test (LLR_2) had a Type I error rate approximately 3 times the nominal rate, and had lower power than LLR_3 for larger differences in asymmetry. The two remaining tests, Fisher's exact test for p (Fep) and the F-test for s_A^2 (F), both exhibited power similar to that of LLR_3 for divergent samples, but also had inflated Type I error rates.

DISTRIBUTION OF ESTIMATES

Distributions of the asymmetry indices from the 200 samples from the power analysis (400 for the base distribution) only occasionally exhibited significant non-normality due to skew or kurtosis (Table 2-4). However,

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Figure 2-6 Estimated power of two-sample tests for differences in asymmetry. Samples of size 50 were generated from Model 2 distribution with $\mu = 10$, $\sigma_L = 0.5$, $\sigma_{a1} = 0.5$, $0.25 \le \sigma_{a2} \le 0.75$.

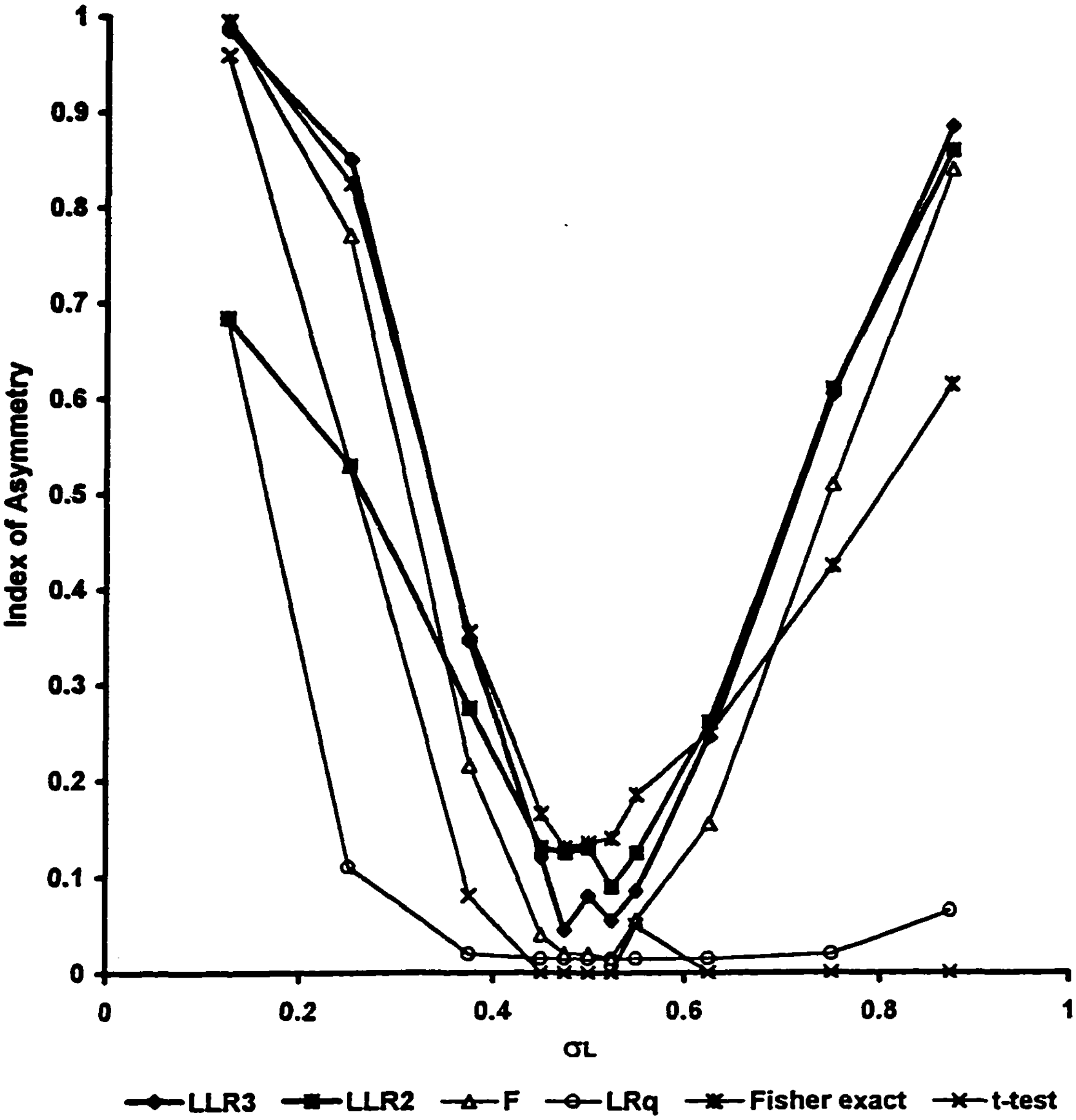

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Table 2-4 Distribution of asymmetry estimates based on samples of size 50 from true full model distributions, $\mu_L = 10.0$, $\sigma_L = 0.5$. Exponents indicate whether the mean was significantly different from the true value of σ_a (m), significant skewness (s), or kurtosis (k). All significance tests are at $\alpha = 0.05$.

	Reduced	Full				
True	Model	Model				
σ_a	$\hat{\sigma}_a$	$\hat{\sigma}_a$	s_A	$\hat{q}$	$\hat{p}$	$\lvert\bar{A}\rvert$
0.125	0.509^m	0.130^s	0.373^m	0.124	0.144^{ms}	0.144^{ms}
0.250	0.551^m	0.248	0.526^m	0.217^m	0.279^m	0.280^m
0.375	0.618^m	0.369	0.658^m	0.292^m	0.407^m	0.417^m
0.450	0.669^m	0.451	0.748^m	0.332^m	0.469^m	0.501^m
0.475	0.683^m	0.484^m	0.791^m	0.352^m	0.506^m	0.548^m
0.500	0.698^m	0.496	0.807^m	0.357^m	0.511^m	0.559^m
0.525	0.704^m	0.509^m	0.825^m	0.363^m	0.520	0.575^m
0.550	0.734^m	0.546	0.870^m	0.381^m	0.551	0.621^m
0.625	0.793^{ms}	0.628	0.975^m	0.415^m	0.598^m	0.714^m
0.750	0.895^m	0.750	1.135^m	0.457^m	0.650^m	0.849^m
0.875	0.995^m	0.863	1.286^m	0.494^m	0.694^m	0.981^{mk}

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three of the five instances of significant skew occurred at the lowest value of σ_a examined (0.125) and were all positive indicating that the lower tail of the distribution of the estimates was truncated at the zero value. $|\overline{A}|$ was the only index to exhibit significant kurtosis, but this single instance may be the result of α-error inflation due to the number of tests conducted. The mean value of $\hat{\sigma}_a$ for the full model differed significantly from the true σ_a for two of the simulations. Except for three instances, all of the other indices were significantly different from the true σ_a.

SENSITIVITY TO COUNTING ERRORS

Measures of fluctuating asymmetry are particularly susceptible to measurement or counting errors. Since the quantity to be measured is a variance, counting errors will inflate the variance and result in a bias to the estimate rather than just a loss of precision. Hubert and Alexander (1995) examined variability of meristic characters of fish (pectoral fin rays, pelvic fin rays, gill rakers, and mandibular pores) and found levels of disagreement of repeated counts to be sufficient to limit the usefulness of the data for fluctuating asymmetry analysis.

Addition of counting errors to samples of size 50 rapidly biased all of the asymmetry indices except for the reduced model $\hat{\sigma}_a$ (sigaR) (Figure 2-7). When errors had a double geometric distribution, a symmetric discrete

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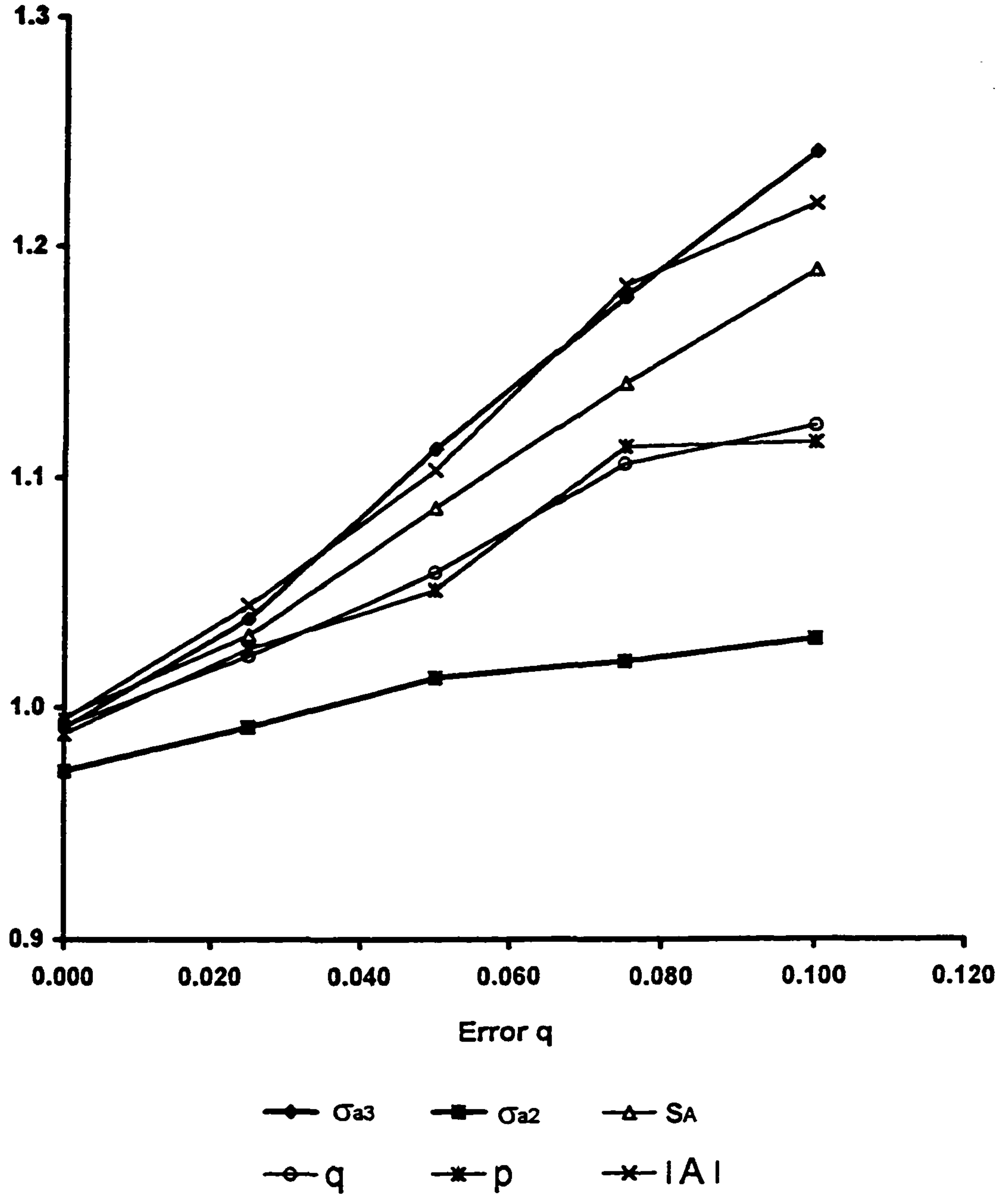
Figure 2-7 Relative bias of asymmetry index values as a function of error probability for bilateral counts. Samples of size 50 were generated from Model 2 distribution with $\mu = 10$, $\sigma_L = 1$, $\sigma_a = 0.5$. Counting errors had a double geometric distribution with $0 \le q \le 0.5$.



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distribution with a mean of 0, the relative bias of the full model $\hat{\sigma}_a$ (sigaF) was approximately 25% at q = 0.1, i.e. counting errors would occur in 10% of the counts. The increase in bias was nearly linear with increasing q. Other indices showed less relative bias as a result of counting errors, but many exhibited considerable bias even without counting errors depending on the true value of σ_a (Figures 2-1 and 2-2). The reduced model $\hat{\sigma}_a$ (sigaR) was least sensitive to counting errors, but was the most biased estimate without counting errors over most of the range of parameters tested.

TESTING DIFFERENCES IN EMPIRICAL DATA

Multiple-sample tests for differences in fluctuating asymmetry were applied to the lake chub data. A Kruskal-Wallis test on $|\bar{A}|$ indicated significant differences in asymmetry among sites for pectoral fin rays, radii on the A scale, and gill rakers (Table 2-5). Jagoe and Haines (1985) found significant differences, using this same test, for pectoral fin rays, and radii on the A and B scales. The likelihood ratio test for the full model (LLR_3) indicated significant ($\alpha = 0.05$) differences among the sites for 5 of the 8 characters tested. Three of the characters were significant at $\alpha = 0.01$. Tests could not be calculated for pelvic fin rays due to the very low amount of variation in the sample from the Penobscot River. The F_{max}-test on s_A^2 from each site produced significant results in 6 of 8 tests,

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Table 2-5 Results of statistical tests for differences in fluctuating asymmetry among three populations of lake chub. * indicates $p < 0.05$. ** indicates $p < 0.01$.

Character	Kruskal-Wallis	LLR_3	F_{max}	G-test
Lateral line scales	3.638	2.349	1.752	9.780**
Scales above lateral line	4.727	14.668**	3.539**	4.051
Scales below lateral line	0.127	3.353	1.652	0.115
Pectoral fin rays	8.840**	5.703*	3.426**	8.851**
Pelvic fin rays	1.790	#	3.083**	2.124
Radii on A scale	6.209*	19.094**	4.535**	1.601
Radii on B scale	5.326	9.569**	2.684**	0.524
Gill Rakers	8.305*	6.518*	2.412**	9.223**

with all 6 being significant at $\alpha = 0.01$. This suggests that the α-error inflation of the F-test observed in the power analysis may also be occurring with the F_{max}-test on the empirical data. The G-test produced a significant result in only 3 of the 8 tests. Only pectoral fin rays and gill rakers exhibited significant results for all four tests.

DISCUSSION

The estimate of σ_a based on the full three-parameter model appears to have many advantages over the previously used indices based on differences in right and left side counts as a measure of fluctuating asymmetry. The full model estimate appears to be much more useful than the reduced two-parameter estimate since, for the empirical data examined, the three parameter model fits the data much better. In addition, the full model $\hat{\sigma}_a$ estimated σ_a well even when the reduced model was the true model for the data because the estimation algorithm was able to get $\hat{\sigma}_L$ to approach 0. However, reduced model $\hat{\sigma}_a$ did not estimate the true σ_a well when the full model was the true model and σ_a:σ_L was < 4.

The primary advantage of the three parameter model $\hat{\sigma}_a$ is that it is nearly unbiased by shifts in the mean liability away from integer values. Swain (1987) and Palmer (1994) clearly explain the problem that a shift from an integer value of the mean liability can cause when the

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actual degree of asymmetry is relatively low. The full model $\hat{\sigma}_a$ is much more stable than are any of the A-based indices.

A second important advantage is that more accurate statistical tests for differences in asymmetry among samples are possible. The likelihood ratio test for $\hat{\sigma}_a$ (LLR_3) was the only test that produced a true nominal α error rate when two samples were drawn from the same population, and also had a relatively high power to detect samples that actually had different asymmetry levels. The power analysis also indicated that with samples as low as 50, only relatively large differences in asymmetry are likely to be detected with any of the tests. The other tests examined were either less powerful and/or had substantial α-error inflation.

$\hat{\sigma}_a$, like all of the difference-based indices, is very susceptible to counting errors. An error in count occuring in only 2.5% of all observations could cause a 5% error in $\hat{\sigma}_a$. The linear increase in bias as the error frequency increases indicates that, to be useful for analysis of fluctuating asymmetry, data sets must be very accurate.

Use of $\hat{\sigma}_a$ as an index of fluctuating asymmetry should improve our ability to estimate the asymmetry of real-world populations from meristic characters. The partitioning of the character variation into components due to variation of the liability (σ_L) and that due to fluctuating asymmetry (σ_a) should allow more accurate

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estimates of fluctuating asymmetry, more powerful tests for differences, and ultimately a better understanding of fluctuating asymmetry and its various causes.

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REFERENCES

Felley, J. 1980. Analysis of morphology and asymmetry in bluegill sunfish (Lepomis macrochirus) in the southeastern United States. Copeia 1980:18-29.

Hilborn, R. and M. Mangel. 1997. The Ecological Detective: confronting models with data. Princeton University Press. Princeton.

Hillier, F. S. and G. J. Lieberman. 1980. Introduction to Operations Research. Third Edition. Holden-Day, Inc. Oakland.

Hubert, W. A. and C. B. Alexander. 1995. Observer variation in counts of meristic traits affects fluctuating asymmetry. North American Journal of Fisheries Management 15:156-158.

Jagoe, C. H. and T. A. Haines. 1985. Fluctuating asymmetry in fishes inhabiting acidified and unacidified lakes. Canadian Journal of Zoology 63:130-138.

Mood, A. M., F. A. Graybill, and D. C. Boes. 1974. Introduction to the theory of statistics. Third Edition. McGraw-Hill, New York.

Palmer, A. R. and C. Strobeck. 1986. Fluctuating asymmetry: measurement, analysis, patterns. Annual Review of Ecology and Systematics 17:391-421.

Palmer, A. R. 1994. Fluctuating asymmetry analyses: A primer, pp.335-364. In T. A. Markow (ed.) Developmental Instability: Its Origins and Evolutionary Implications, Kluwer, Dordrecht, Netherlands.

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Palmer, A. R. and C. Strobeck. 1992. Fluctuating asymmetry as a measure of developmental stability: Implications of non-normal distributions and power of statistical tests. Acta Zoologica Fennica 191:55-70.

Sokol, R. R. and F. J. Rohlf. 1993. Biometry. Third Edition. W. H. Freeman, San Francisco.

Swain, M. W. 1987. A problem with the use of meristic characters to estimate developmental stability. The American Naturalist 129:761-768.

Van Valen, L. 1962. A study of fluctuating asymmetry. Evolution 16:125-142.

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CHAPTER 3

A RE-EXAMINATION OF FLUCTUATING ASYMMETRY IN MERISTIC CHARACTERS IN FISH USING THE THREE-PARAMETER DEVELOPMENTAL MODEL

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ABSTRACT

Fluctuating asymmetry has been used successfully in many taxa to indicate developmental instability resulting from contaminant or genetic stress. In fish, which have been analyzed primarily using meristic characters, fluctuating asymmetry has had mixed results. While some studies have demonstrated higher asymmetry in population living in presumably more stressful conditions, others have found no differences in levels of fluctuating asymmetry.

Data from four studies were reanalyzed using a three-parameter developmental model and associated statistical tests, and compared to the analyses originally reported.

For Sonoran topminnow, the three parameter model found significant differences in fluctuating asymmetry among three populations in four of seven characters examined, compared to three of seven in the analysis originally conducted; however, the conclusion that asymmetry increased as heterozygosity decreased was not changed. The original metric used, the standard deviation of the left-right differences, may have been biased by violation of normality assumptions.

Five meristic characters examined in 8 hatchery strains of rainbow trout showed not significant relationship with fluctuating asymmetry measured by proportion of individuals asymmetric or by the three-parameter model.

Partially plated morphs of three Pacific coast three-spine stickleback populations exhibited higher fluctuating

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asymmetry than did the complete or low plate morphs. Although the three-parameter model did not detect any differences among populations within morph type, differences were found using the proportion asymmetric and mean absolute difference. The presence of a few outlier values in the data sets were found to have substantial effects on the level of α-error in statistical tests.

Bluegills collected from low pH lakes were not found to consistently more asymmetric than those from circumneutral lakes. The three-parameter model found differences among lake groups for two of three characters examined, but one of those indicated significantly higher asymmetry in the circumneutral lake group.

Overall, use of the three-parameter model did not change the conclusions of the original investigators. The failure to find strong relationships of heterozygosity and pH with fluctuating asymmetry could be due to a truly weak relationship, or to other factors such as low sample sizes, errors in character counts, or use of mixed-age samples.

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INTRODUCTION

Fluctuating asymmetry is the type of asymmetry that occurs when a measurable or countable bilateral characteristic varies randomly between one side of the organism and the other. It is measured in individual organisms as the absolute or relative difference between the characteristic on the right and left sides. Differences of measurable characteristics generally vary according to a normal distribution (Van Valen 1962), with a mean value 0. For populations, fluctuating asymmetry is measured by a statistic of the individual differences, usually as some estimate of the variance of the individual differences (Palmer and Strobeck 1992).

In the last 25 years, fluctuating asymmetry has been examined as a potential indicator of sublethal stress in many animal species. Many studies have shown that increased levels of stress during development, whether due to environment (Leary et al. 1992), toxic chemicals (Valentine and Soulé 1973; Clarke 1992; Graham et al. 1993), or genetic factors (Clark 1992, Quattro and Vrijenhoek 1989; Vrijenhoek and Lerman 1982), result in higher levels of asymmetry that can be detected with statistical techniques designed to identify differences in variance of normal distributions.

Valentine et al.(1973) were among the first to examine fluctuating asymmetry in fish populations. They demonstrated trends in pectoral fin asymmetry in Pacific coast marine fish populations along a pollution gradient.

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Subsequently, fluctuating asymmetry was examined in many other fish populations; however, many studies were not able to link potential stresses with degree of asymmetry. Ames et al. (1979) were unable to detect differences in fluctuating asymmetry among largemouth bass, *Micropterus salmoides*, bluegill, *Lepomis macrochirus*, and redbreast sunfish, *Lepomis auritus* from heated and unheated reservoirs, although fish from a pond heavily contaminated with mercury were more asymmetric. Felley (1980) found no increase in asymmetry for hybrids of two subspecies of bluegills. Carline and Lawal (1985) concluded that fluctuating asymmetry was not a useful indicator of pollution stress for Ohio and Wisconsin populations of yellow perch, *Perca flavescens*. Asymmetry of some, but not all, bilateral characteristics of brook trout, *Salvelinus fontinalis*, white suckers, *Catostomus commersoni*, and lake chub, *Couesius plumbeus*, were weakly associated with reduced pH levels (Jagoe and Haines 1985). Wiener and Rago (1987) could find no increase in fluctuating asymmetry associated with low pH stress in bluegills.

There were also studies in which fluctuating asymmetry was demonstrated to increase with a potential stressor. Graham and Felley (1985) found increased fluctuating asymmetery in a zone of hybridization between two species of sunfish. Vrijenhoek and Lerman (1982) demonstrated a decrease in fluctuating asymmetry with increasing heterozygosity among *Poeciliopsis monacha* populations, but

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no change in fluctuating asymmetery for a clonal hybrid population. Three isolated populations of Sonoran topminnow, Poeciliopsis occidentalis, had fluctuating asymmetry varying inversely with heterozygosity (Quattro and Vrijenhoek, 1989). Zakharov and Zyuganov (1980) determined that polymorphic populations, indicative of intergradation of freshwater and marine populations, of three-spine sticklebacks, Gasterosteus aculeatus, had higher fluctuating asymmetry than the monomorphic isolated populations, suggestive of stress from loss of coadaptation.

Analyses of fluctuating asymmetry in other major groups of organisms have encountered similar inconsistent results. In a review of fluctuating asymmetry studies, Palmer and Strobeck (1992) concluded that the multitude of statistical methods that have been used in data analysis have resulted in inconsistent and incomparable results. They found that the statistical precision and power of some of the common measures and tests of asymmetry for morphometric (measurable) characters varied substantially and suggested some standardized methods to improve future studies.

The variability of study results may also be due to the complexity of factors that determine the level of fluctuating asymmetry. Although heterozygosity may in general reduce fluctuating asymmetry, Vrijenhoek and Lerman (1982) found that populations with a heterozygosity of about 4% were no more asymmetric than populations with higher levels of heterozygosity. The explanation for the

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differences in the asymmetry of hybrid sunfish populations may be coadaptation of gene complexes. The bluegill populations of Felley (1980) had hybridized long ago and had sufficient evolutionary time to redevelop coadaptation in the hybrid population. In contrast, the populations studied by Graham and Felley (1985) had hybridized as the result of man-made modifications to the environment and had not had sufficient time to re-establish coadaptation, thus hybrids were more asymmetric than either of the parental species.

Finally, experimental design issues may contribute to the inconsistency of the results. Typically, sample sizes from populations have been on the order of 50 individuals. Since many meristic characters in fish are determined early in the first year of life, variability of environmental conditions among years could act to mask genetically-based differences in fluctuating asymmetry among populations. Counting errors will bias asymmetry estimates, but the effects on fluctuating asymmetry analyses are not widely understood (Markow 1994).

For meristic characters there is also a methodology problem that can bias estimates of fluctuating asymmetry. The bias arises when the mean value of the character being counted departs from an integer value for some groups but not for others (Swain 1987). The bias is more severe for characters with high developmental stability (Swain 1987, Palmer 1994), which can lead to inaccurate results when comparing populations that have different character means.

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In Chapter 2 I developed a new set of fluctuating asymmetry statistics that are far less biased by changes in the character mean than the statistics traditionally used for meristic characters, and also presented likelihood ratio tests for comparing fluctuating asymmetry across populations. In this chapter I apply the new methods to previously collected data to examine whether the new index and associated statistical tests result in improved understanding of fluctuating asymmetry and its causes.

METHODS

Empirical data on asymmetry of meristic characters were obtained for samples of fish from previously reported studies, and from additional unpublished data. Sonoran topminnow (*Poeciliopsis occidentalis*) from Quattro and Vrijenhoek (1989), hatchery strains of rainbow trout (*Oncorhynchus mykiss*) from Leary et al. (1992), and published and unpublished data on threespine sticklebacks (*Gasterosteus aculeatus*) from Bell (1982) were used to examine the relationship between fluctuating asymmetry and heterozygosity. Data on bluegill (*Lepomis macrochirus*) populations from low and circumneutral pH lakes were obtained from Weiner and Rago (1987).

Three parameters (μ, σ_L, σ_a) based directly on a conceptual model of development (Chapter 2) were estimated for each data set. The character state of a bilateral meristic character (Y_{ij}) can be described as the realized value of the liability, the underlying normally distributed tendency for a meristic character (Swain 1987), rounded to the nearest integer. The realized liability (X_{ij}) is the sum of three continuous random variables:

$$X_{ij} = \mu + \delta_i + e_{ij}$$

where μ = mean of liability for the population

δ_i = Normal$(0,\sigma_L^2)$ deviation from mean liability for organism i

e_{ij} = Normal$(0,\sigma_a^2)$ deviation on side j of organism i from developmental instability

This three-parameter mathematical model accounts separately for variation of the character value among organisms with the parameter σ_L, and for developmental disruptions that result in fluctuating asymmetry using σ_a. The conceptual developmental model described by Swain (1987) is a special case of this model in which $\sigma_L \equiv 0$.

The maximum likelihood estimates of the model parameters μ, σ_L, and σ_a, were found using an iterative gradient search algorithm (Chapter 2). Parameter estimates were calculated for both the two-parameter model ($\sigma_L \equiv 0$) and the full three parameter model. The negative log of the likelihood (NLL) was used to compare the fit of these models to the data. A generalized likelihood ratio test, based on the likelihood functions for the two models (Chapter 2), was used to test whether the three parameter model was significantly better than the reduced model. Since the three parameter model was nearly always significantly

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better; only the results of the three-parameter model are given.

In addition, the index of asymmetry used by the original authors was calculated for comparison. The previous studies used several different indices, but all were based on the difference in right and left side character counts:

$$A_i = Y_{ir} - Y_{il}$$

where Y_{ir} = count on the right side of the fish i

Y_{il} = count on the left side of the fish i

The distribution of A_i for each characteristic was checked for departure from a normal (μ=0) distribution using a t-test and tests for skewness (g_1) and kurtosis (g_2) (Palmer 1994). Tests were conducted at a single test $\alpha = 0.05$. The indices based on A were: s_A, the standard deviation of the difference; $|\bar{A}|$, the mean of the absolute difference, and $\hat{p}$, the proportion of the sample that is asymmetric.

Type I and Type II statistical errors were evaluated using the stickleback data, due to the large sample sizes available for this species. Type I error rates were determined by repeatedly drawing two samples of 50 organisms (with replacement) from a singe data set (low plate morphs from Brush Creek), and testing whether the indices of fluctuating asymmetry were signficantly different ($\alpha = 0.05$) for the two samples. Type II error was determined by repeatedly drawing samples from two different populations (low plate morphs from Brush Creek and Garcia River) and

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testing whether the indices were significantly different for a range of sample sizes from 50 to 200 for each population.

RESULTS

Sonoran topminnow

Quattro and Vrijenhoek (1989) found significant differences in fluctuating asymmetry among three populations of Sonoran topminnow for 3 of the 7 characters they examined using the Kruskal-Wallis test on s^2_A, the variance of the A_i values (Table 3-1). In general, the degree of fluctuating asymmetry was negatively related to the amount of heterozygosity at 25 loci. The most heterozygous population (Sharp Spring; mean H = 3.7%) typically had the lowest fluctuating asymmetry, while the most homozygous (Monkey Spring; mean H = 0.0%) was usually the most asymmetric. They also demonstrated that fitness measures (survival, growth, and fecundity) were positively related to heterozygosity.

Of the 63 statistical tests conducted to examine normality of the A_i distributions (mean = 0, skewness, and kurtosis for each of the 3 populations for 7 characters), significant skewness was observed 6 times and significant kurtosis 9 times (Table 3-1), even with the relatively low samples sizes employed (Monkey Spring n = 25; Tule Spring n = 49, Sharp Spring n = 35).

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Table 3-1. Asymmetry parameter estimates $\hat{\mu}$, $\hat{\sigma}_L$, $\hat{\sigma}_a$, and s_A for Sonoran topminnow collected from three remnant populations in Arizona, listed in order of increasing heterozygosity. Characters examined were lateral line scales (LLS), dorsolateral scale count (DLS), pectoral fin rays (PFR), pelvic fin rays (VFR), dentary outer teeth (DOT), and premaxillary outer teeth (POT). Mean heterozygosity (H) and maximum sample size for each population are also given. Data from Quattro and Vrijenhoek (1989). Superscript s indicates significant skew of the A_i distribution; k indicates significant kurtosis.

	Monkey Spring				Tule Spring				Sharp Spring			
Mean H (%)	0.0				1.5				3.7			
Maximum N	25				49				35			
Character	$\hat{\mu}$	$\hat{\sigma}_L$	$\hat{\sigma}_a$	s_A	$\hat{\mu}$	$\hat{\sigma}_L$	$\hat{\sigma}_a$	s_A	$\hat{\mu}$	$\hat{\sigma}_L$	$\hat{\sigma}_a$	s_A
LLS*	28.4	0.39	0.434	0.748	28.5	0.50	0.346	0.623^{k}	28.8	0.46	0.133	0.378^{k}
DLS	9.5	0.81	0.529	0.849	9.3	1.13	0.631	0.979	8.5	1.15	0.358	0.655
PFR*	14.2	0.30	0.260	0.529^{k}	14.1	0.31	0.110	0.319^{k}	14.2	0.26	0.028	0.169^{sk}
VFR*	6.8	0.30	0.549	0.825^{sk}	6.4	0.62	0.653	0.990^{sk}	6.9	0.05	0.358	0.507^{sk}
DOT	11.3	1.96	0.735	1.114^{k}	13.2	1.61	0.826	1.229^{sk}	11.5	2.96	0.928	1.363^{sk}
POT	11.1	1.90	0.772	1.166	13.4	1.55	0.632	0.979	12.3	1.81	0.512	1.907

* indicates significant ($p < 0.05$) differences among populations reported by authors.

The maximum likelihood estimates of asymmetry, $\hat{\sigma}_a$, from the three-parameter model were lower than the standard deviation of the A_i (s_A), as was demonstrated from the simulation results in Chapter 2. For pectoral fin rays, which had a very low amount of variation, $\hat{\sigma}_a$ converged with s_A, also as demonstrated with the simulations. The likelihood ratio test on $\hat{\sigma}_a$ indicated significant differences among the three populations in four of six characters tested (Figure 3-1). No estimates were calculated for scales above the lateral line because only one individual was asymmetric in two of the populations and there were no asymmetric individuals in the other. Overall, $\hat{\sigma}_a$ decreased as heterozygosity increased, but dentary outer teeth showed the opposite trend, and two characters, dorsolateral scale count and pelvic fin rays, had highest asymmetry in the Tule Spring population which was intermediate in heterozygosity. There was no clear relationship of interindividual variation $\hat{\sigma}_L$ with heterozygosity (Figure 3-2).

Rainbow Trout

Leary et al. (1992) examined fluctuating asymmetry of five meristic characters with respect to heterozygosity in 20 strains of rainbow trout. They found no significant correlation between mean asymmetry of a strain (measured as the mean number of asymmetric characters) and mean

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Figure 3-1 $\hat{\sigma}_a$ for Sonoran topminnow collected from three remnant populations in Arizona as a function of mean heterozygosity. Characters examined were lateral line scales (LAS), dorsolateral scale count (DC), pectoral fin rays (PFR), pelvic fin rays (VFR), dentary outer teeth (DOT), and premaxillary outer teeth (POT). Bold lines indicate significant differences in $\hat{\sigma}_a$ among populations.

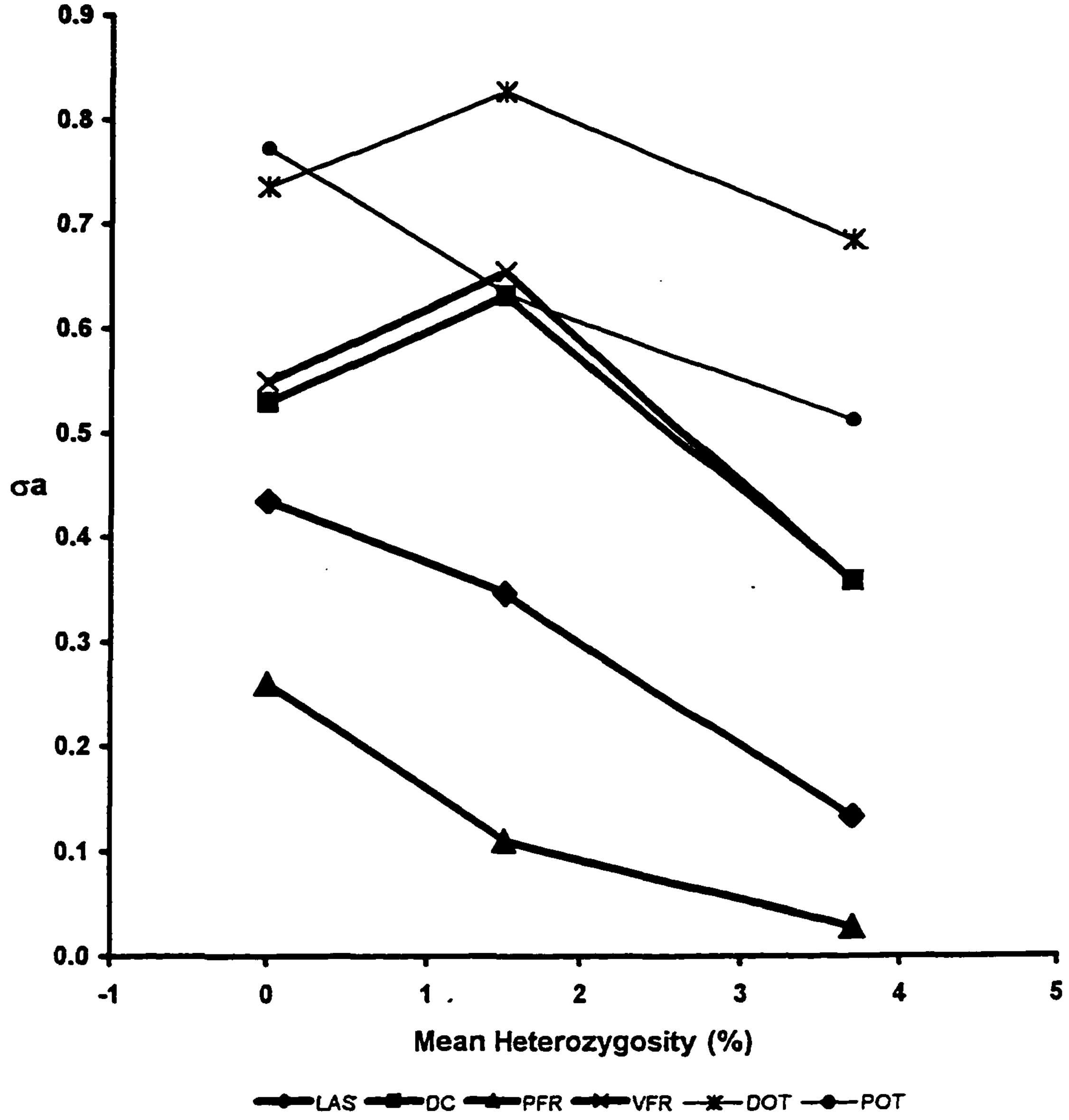

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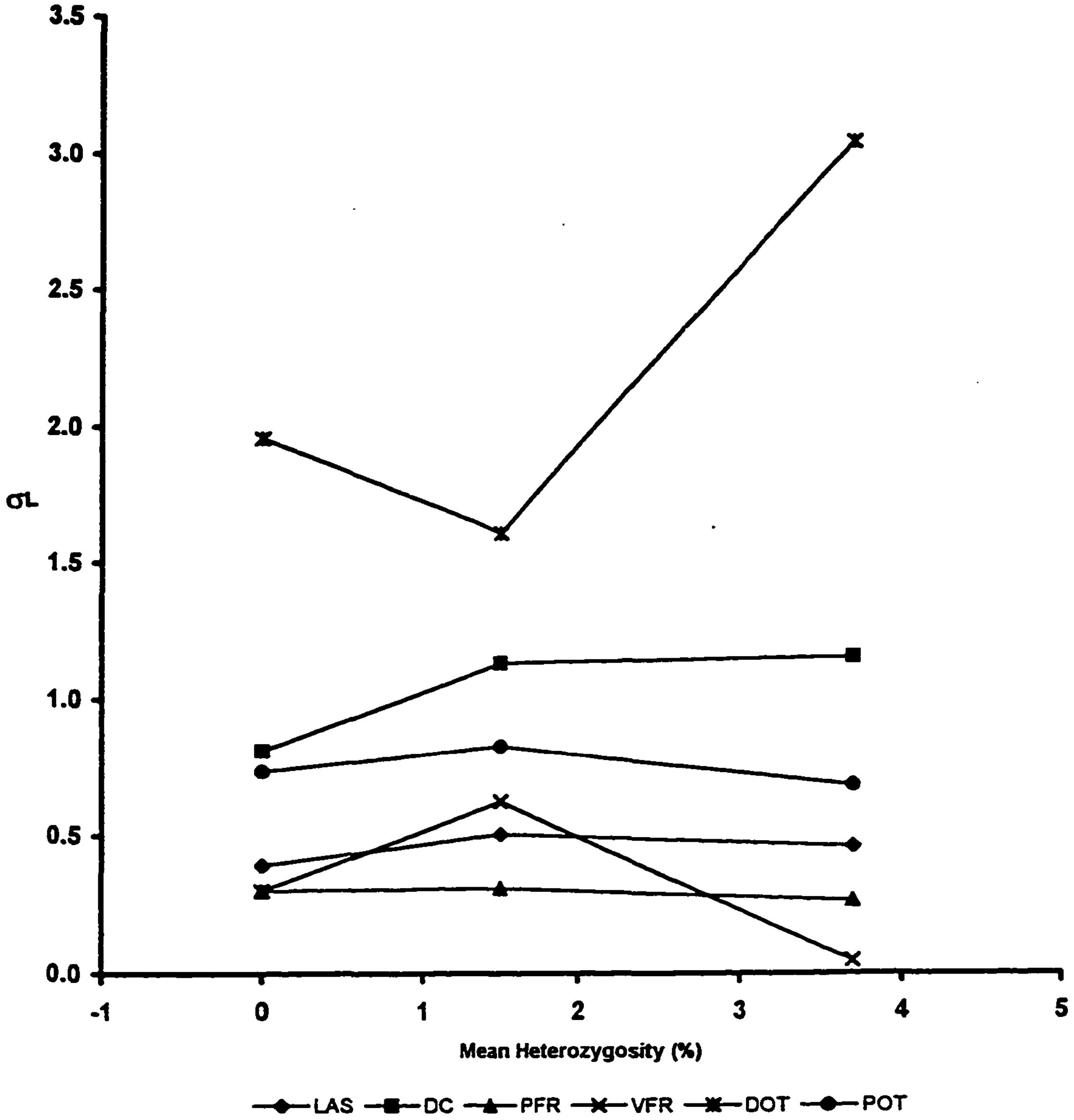
Figure 3-2 $\hat{\sigma}_L$ for Sonoran topminnow collected from three remnant populations in Arizona as a function of mean heterozygosity. Characters examined were lateral line scales (LAS), dorsolateral scale count (DC), pectoral fin rays (PFR), pelvic fin rays (VFR), dentary outer teeth (DOT), and premaxillary outer teeth (POT).



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heterozygosity at 40 protein coding loci. When examined on an individual fish basis (number of asymmetric characters) they found significant correlations with heterozygosity for only 4 out of 20 strains, although 18 of the 20 estimates of r were negative.

A subset of 8 of the strains from Leary et al. (1992) indicated no significant departures from a mean $A_i = 0$ (out of 40 tests), 10 instances of significant skewness, and 23 instances of significant (lepto)kurtosis. Pectoral fin rays (8), upper gill rakers (6) and pelvic fin rays (5) were the most leptokurtotic characters.

The model-based estimate of fluctuating asymmetry, $\hat{\sigma}_a$, was numerically very similar to p (Tables 3-2 and 3-3), although sometimes larger than p and sometimes smaller. The correlation of fluctuating asymmetry with the mean population heterozygosity was not significant for either $\hat{\sigma}_a$ or p (Table 3-4). In addition, the estimate of between-individual variation, $\hat{\sigma}_L$, was not significantly correlated with heterozygosity. Upper gill rakers was the character closest to the expected pattern of a positive relation between $\hat{\sigma}_L$ and heterozygosity, and a negative relationship between $\hat{\sigma}_a$ and heterozygosity (Figure 3-3). Likelihood ratio tests for differences in $\hat{\sigma}_a$ among strains were significant for pectoral fin rays and pelvic fin rays, but the correlation analysis indicated a positive rather than negative relationship with heterozygosity.

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Table 3-2 Asymmetry parameter estimates $\hat{\mu}$, $\hat{\sigma}_L$, $\hat{\sigma}_a$, and p for mandibular pores, pectoral fin rays, and pelvic fin rays for hatchery strains of rainbow trout, listed in order of increasing heterozygosity. Data from Leary et al. (1992). Superscript s indicates significant skew of the A_i distribution; k indicates significant kurtosis.

Strain	H	Maximum sample size	Mandibular pores				Pectoral fin rays				Pelvic fin rays			
			μ	$\hat{\sigma}_L$	$\hat{\sigma}_a$	p	μ	$\hat{\sigma}_L$	$\hat{\sigma}_a$	p	μ	$\hat{\sigma}_L$	$\hat{\sigma}_a$	p
Kamloops	3.9	50	8.4	0.32	0.57	0.62	14.1	0.49	0.17	0.18^{k}	9.7	0.18	0.08	0.10^{k}
Erwin	4.7	59	7.3	0.43	0.64	0.58	14.2	0.61	0.19	0.22^{k}	10.1	0.29	0.18	0.14^{sk}
McConaughy-B	4.9	50	7.8	0.61	0.39	0.41	15.2	0.66	0.36	0.26^{k}	10.0	0.28	0.17	0.14^{sk}
Arlee-A	6.5	50	7.9	0.46	0.65	0.58	14.4	0.58	0.32	0.28^{k}	9.9	0.33	0.16	0.16^{k}
Shepherd Hills	6.8	50	7.6	0.53	0.60	0.54^{sk}	13.7	0.78	0.46	0.44	10.1	0.27	0.10	0.08^{k}
McConaughy-A	7.3	50	8.2	0.44	0.62	0.62^{s}	15.2	0.42	0.37	0.30^{k}	10.3	0.39	0.10	0.10^{k}
Shasta	8.5	67	8.1	0.68	0.55	0.60	14.3	0.58	0.36	0.37	9.8	0.26	0.29	0.22^{sk}
Bennet Spring	8.9	50	7.7	0.56	0.56	0.48^{k}	14.2	0.44	0.24	0.26	9.8	0.53	0.15	0.14^{k}

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Table 3-3 Asymmetry parameter estimates $\hat{\mu}$, $\hat{\sigma}_L$, $\hat{\sigma}_a$, and p for upper and lower gill rakers for hatchery strains of rainbow trout, listed in order of increasing heterozygosity. Data from Leary et al. (1992). Superscript s indicates significant skew of the A_i distribution; k indicates significant kurtosis.

		Maximum sample	Upper gill rakers				Lower gill rakers			
Strain	H	size	μ	$\hat{\sigma}_L$	$\hat{\sigma}_a$	p	μ	$\hat{\sigma}_L$	$\hat{\sigma}_a$	p
Kamloops	3.9	50	8.0	0.36	0.21	0.22^{k}	11.2	0.44	0.39	0.34^{sk}
Erwin	4.7	59	9.2	0.53	0.28	0.32	11.4	0.52	0.60	0.36^{sk}
McConaughy-B	4.9	50	8.5	0.40	0.22	0.27	10.8	0.54	0.46	0.46
Arlee-A	6.5	50	8.8	0.52	0.30	0.24^{sk}	11.7	0.58	0.56	0.54
Shepherd Hills	6.8	50	8.9	0.56	0.13	0.14^{k}	11.2	0.59	0.38	0.40
McConaughy-A	7.3	50	8.3	0.48	0.15	0.18^{k}	10.6	0.47	0.40	0.42
Shasta	8.5	67	9.0	0.55	0.29	0.25^{sk}	10.6	0.45	0.42	0.43
Bennet Spring	8.9	50	8.2	0.38	0.16	0.18^{sk}	10.7	0.41	0.48	0.58

Table 3-4. Pearson correlation of fluctuating asymmetry measures with mean population heterozygosity for eight strains of rainbow trout. Data from Leary et al. (1992). None of the correlations are significant at $\alpha = 0.05$.

	Heterozygosity correlation with		
Character	p	$\hat{\sigma}_a$	$\hat{\sigma}_L$
Mandibular pores	-0.027	0.610	0.142
Pectoral fin rays	0.571	-0.236	0.413
Pelvic fin rays	0.381	0.679	0.384
Upper gill rakers	-0.478	0.263	-0.200
Lower gill rakers	0.637	-0.320	-0.161

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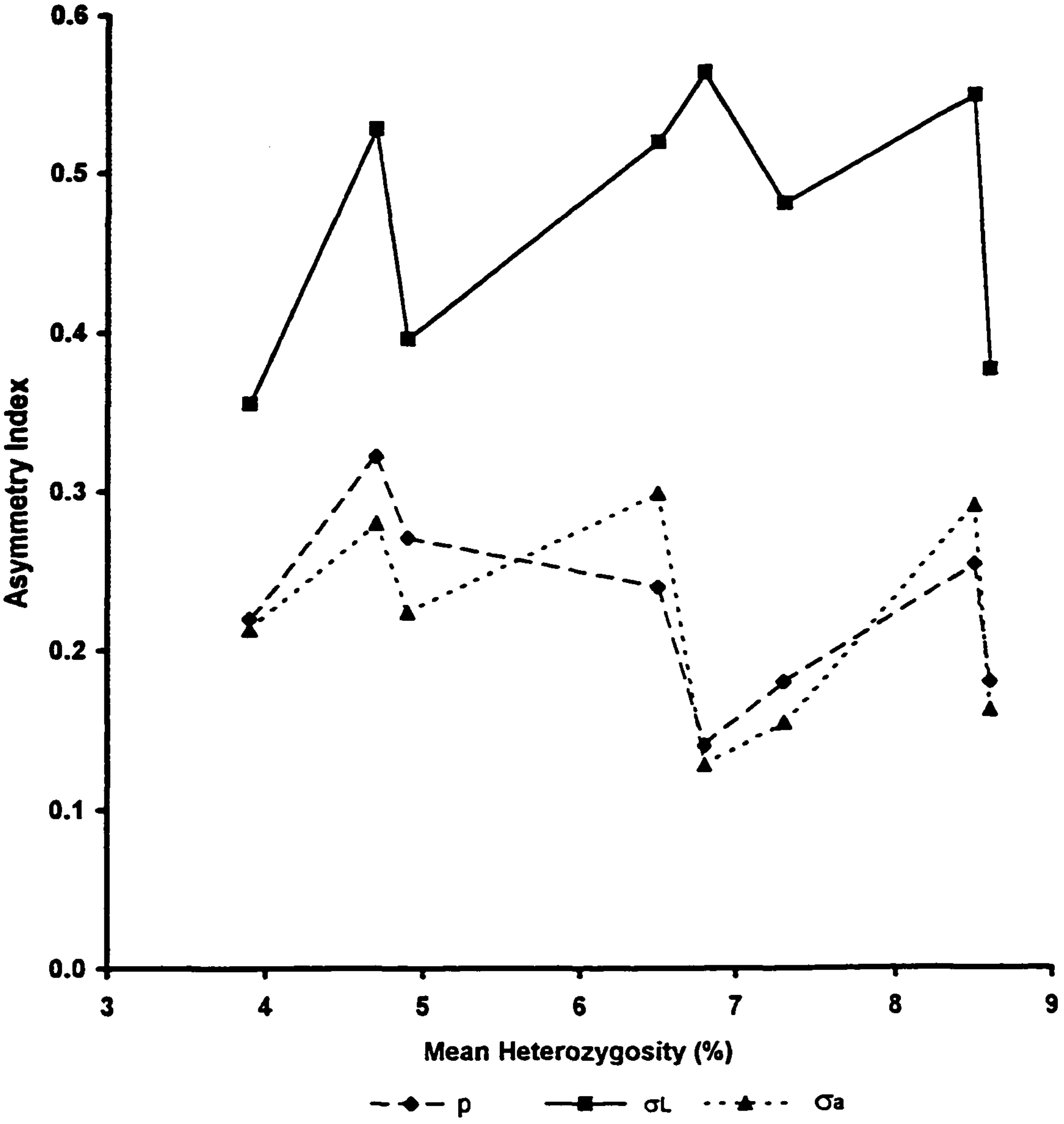
Figure 3-3 σ_a, σ_L and p for upper gill rakers for eight strains of rainbow trout as a function of mean heterozygosity.



Three-spine stickleback

Bell (1982) has collected data on the lateral plate morph patterns of threespine stickleback populations on the Pacific coast. Populations may contain one, two, or all three morphs, depending on the environmental conditions and habitats available to the population. Complete morphs have lateral plates lining the abdomen all the way posterior to the caudal peduncle. Typically there are 30 or more plates on each side. Low plate morphs contain plates only on the abdomen, usually less than 10 plates on each side. Partial morphs have plates on both the abdomen and tail, but have a gap that is unplated between these areas. Partial morphs typically have between 10 and 30 plates per side.

Previous work on G. aculeatus in the White Sea has shown that variability and fluctuating asymmetry of the partial morphs is higher than in the complete and low morphs (Zakharov and Zyuganov 1980). I used data that Bell collected from three Pacific coast systems to see whether northern California populations exhibited the pattern shown in the White Sea, and to test whether fluctuating asymmetry differences existed among populations.

The three northern California populations matched the pattern previously seen in the White Sea in that the partial morphs were both more variable and more asymmetric than either the complete or low morphs as indicated by $\hat{\sigma}_L$ and $\hat{\sigma}_a$ (Table 3-5). Although the complete morphs from Brush Creek had a $\hat{\sigma}_L$ over twice that of the Garcia River fish, the

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Table 3-5. Asymmetry parameter estimates $\hat{\mu}$, $\hat{\sigma}_L$, $\hat{\sigma}_a$, $|\bar{A}|$, and $\hat{p}$ for complete, partial, and low lateral plate morphs of threespine sticklebacks. N = sample size. Superscripts denote significant differences ($p < 0.05$). From published and unpublished data from M. A. Bell.

Morph	Population	N	$\hat{\mu}$	$\hat{\sigma}_L$	$\hat{\sigma}_a$	$\lvert\bar{A}\rvert$	$\hat{p}$
Complete	Brush Creek	131	32.536	1.153	0.380	0.145^a	0.084^a
	Garcia River	145	32.771	0.537	0.374	0.386^b	0.366^b
Partial	Brush Creek	145	21.572	5.331	1.481	1.517	0.793
	Garcia River	32	16.765	7.440	1.184	1.281	0.719
Low	Brush Creek	370	6.753	0.956	0.472	0.378	0.354
	Garcia River	108	6.373	0.844	0.555	0.463	0.380
	Big Lagoon	138	6.661	1.131	0.522	0.464	0.413

estimates of $\hat{\sigma}_a$ were nearly identical. For the low plate morphs, both the $\hat{\sigma}_L$ and $\hat{\sigma}_a$ values were very similar between Brush Creek and Garcia River, and also with Big Lagoon where the population was essentially monomorphic.

Although none of the comparisons between populations within a morph indicated significant differences in fluctuating asymmetry using $\hat{\sigma}_a$, two other asymmetry measures were also calculated for comparison. For the complete morphs, both the proportion asymmetric and $|\overline{A}|$ indicated significant differences between the Brush Creek and Garcia River populations. Although the low plate morphs indicated no significant differences using either of these parameters, the order of the fluctuating asymmetry scores varied from that obtained using $\hat{\sigma}_a$.

Estimates of Type I error (rejection of a true H_0: samples are from the same population) indicate severely inflated error rates for all of the tests examined. The proportion of iterations that rejected H_0 (at a nominal α = 0.05) ranged from 0.06 for the G-test to 0.40 for the likelihood ratio test (LLR3). Even though these estimates are based on only 100 replications of the sampling, Type I errors for LLR3 and the F-test were clearly well above the nominal rates. The reason for the high α-error rates may lie with the distribution of the lateral plate counts. Although the majority of the counts appear to arise from a bivariate normal distribution, there are several observations (i.e. fish) that are relatively extreme

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outliers (Figure 3-4). To test whether the five extreme data values (any count > 10) were the reason for the inflated α-error, those values were removed from the data set and the iterative resampling exercise was repeated, with a result that α-error rates were much reduced. However, the likelihood ratio test (LLR3), the F-test, and the Kruskal-Wallis test all indicated significance approximately 10% of the time rather than the expected 5% based on 600 iterations (indicated as the "0" sample size values on Figure 3-5). The G-test for the proportion of individuals that were asymmetric had approximately the correct error rate.

Power of the statistical tests, the converse of the Type II error rate, when the samples were drawn repeatedly from the Brush Creek and Garcia River samples with outlier values removed from both data sets, was highest for LLR3 and the F-test. Both of these tests exhibited significance approximately 50% of the time at a sample size of 50, improving to approximately 90% at a sample size of 200 (based on 250 replications at each sample size). The true asymmetry values calculated for the edited data sets were $\hat{\sigma}_a = 0.344$, $s_A = 0.624$, $|\bar{A}| = 0.362$, and $\hat{p} = 0.348$ for Brush Creek, and $\hat{\sigma}_a = 0.517$, $s_A = 0.820$, $|\bar{A}| = 0.467$, and $\hat{p} = 0.383$ for Garcia River, thus the differences in asymmetry were not large. The power curves for LLR3 and the F-test are probably not significantly different from each other given that they are based on 250 replications at each sample size.

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Figure 3-4 Distribution of right and left side lateral plate counts for low plate morphs of three-spine stickleback from Brush Creek. Dark bars (right or left count > 10) were removed from the data set prior to the power analysis.

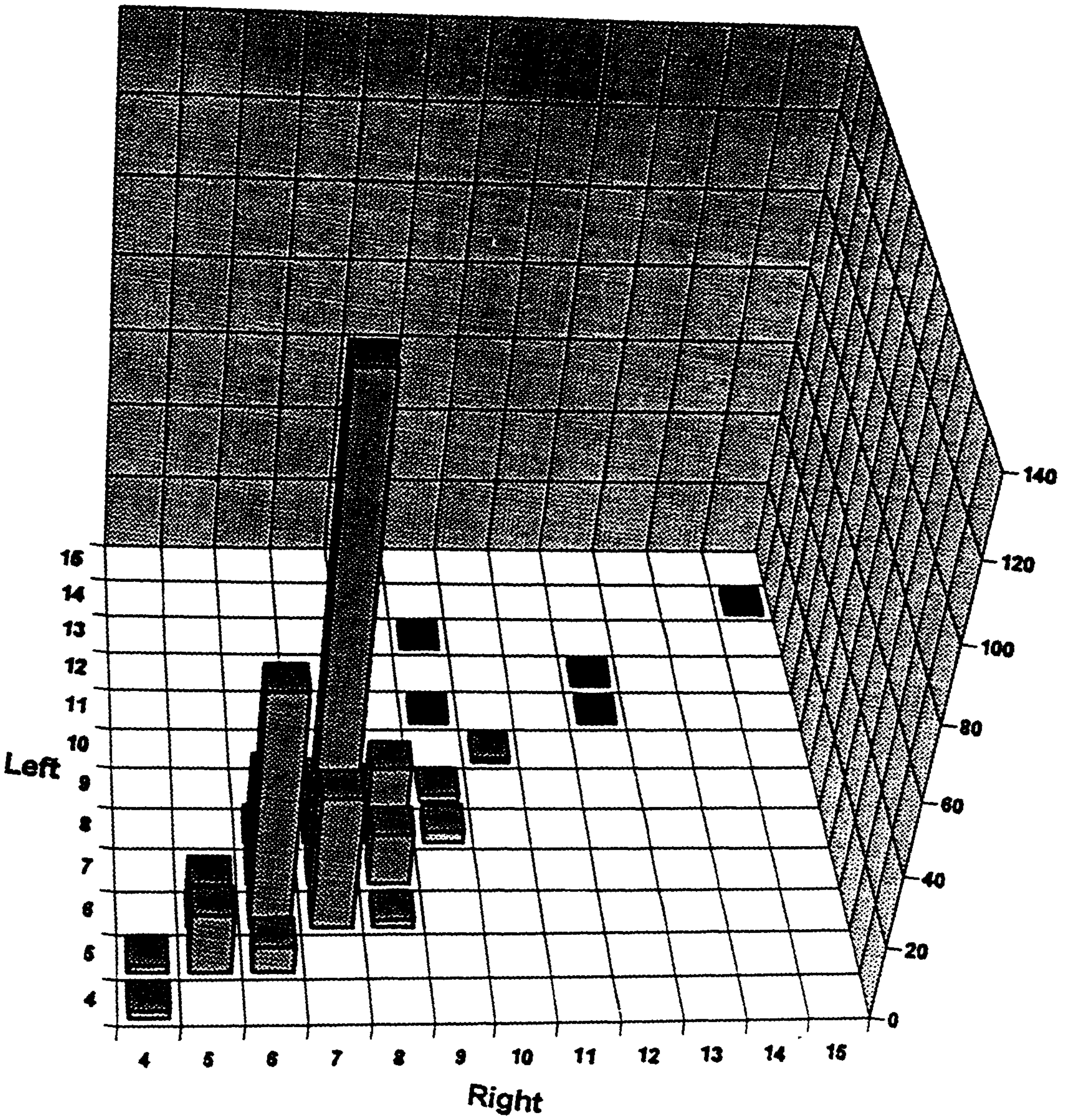

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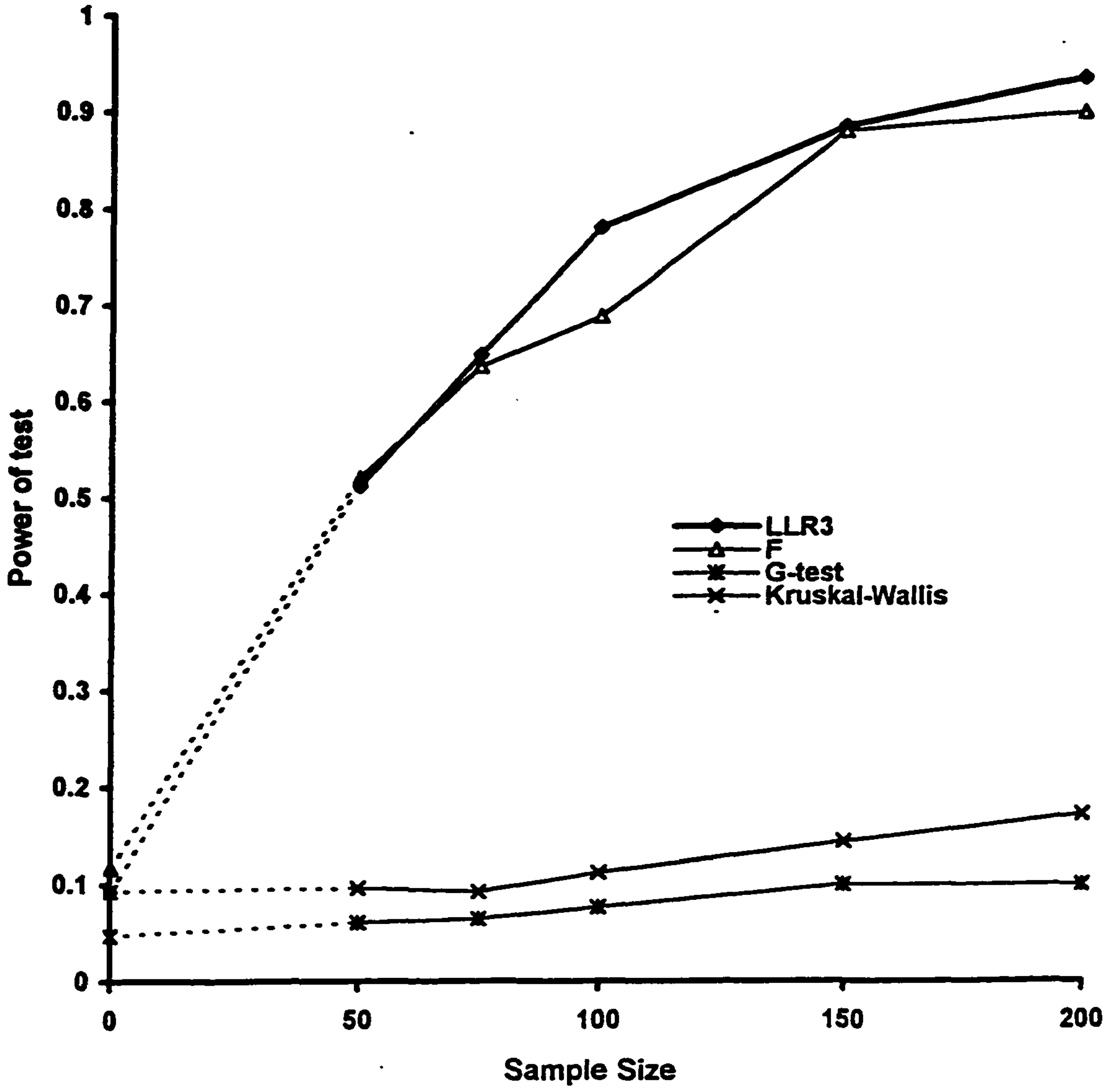
Figure 3-5 Power of statistical tests for detecting differences between low plate morph samples from Brush Creek and Garcia River as a function of sample size. Sample size 0 represents the Type I error rate for testing two random samples of size 50 from the Brush Creek population. Outlier samples (any count > 10) were removed from both populations prior to analysis.



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The G-test and Kruskal-Wallis test both had much lower power, and power increased very little with larger samples.

Bluegill

Weiner and Rago (1987) examined fluctuating asymmetry, measured as both the mean $|\overline{A}|$ and variance $s^2_{|A|}$ of the absolute differences, in bluegill populations in Wisconsin lakes in an effort to relate fluctuating asymmetry to pH. They collected bluegills from five lakes with pH ≤ 6.0 and from six lakes with pH ≥ 6.7. Mean calcium concentration in the low pH lakes was 1.5 mg/l, and in the circumneutral lakes 6.4 mg/l. Characters examined were the number of pored lateral line scales, pectoral fin rays, and gill rakers on the outer arch.

The two measures of fluctuating asymmetry were highly correlated for all three characters. Weiner and Rago tested the $|A_i|$ distributions for each of the lakes against a Poisson distribution with mean equal to the overall mean, the mean for low pH lakes, and the mean for circumneutral lakes using the Kolmogorov-Smirnov test and found no significant differences in the 99 comparisons. Using $s^2_{|A|}$ they found significant relationships between lake category (low or circumneutral pH) and fluctuating asymmetry for gill rakers, but not for pectoral fin rays or pored lateral line scales.

Prior to completing the reanalysis for these data, two extreme values of the lateral line scale A_i were found in the data and discussed with one of the original investigators (PJR). It was determined that these two extreme differences were likely to be the result of data entry error that could not at this date be resolved by checking the original data sheets. These two observations, one for Hildebrand Lake and one for Hixon Lake, were removed from the data.

Examination of the A_i values for normality indicated no significant difference from a mean of 0, 8 instances of significant skewness (5 for pectoral fin rays), and 18 instances, including all 11 tests for pectoral fin rays, of significant kurtosis. All 18 significant kurtosis tests indicated leptokurtosis.

The model-based estimate of fluctuating asymmetry, $\hat{\sigma}_a$, was numerically very similar to $|\bar{A}|$ (Table 3-7), although sometimes larger than $|\bar{A}|$ and sometimes smaller. Likelihood ratio tests for differences in σ_a between the low pH and the circumneutral lakes indicated significant differences for pored lateral line scales (pooled $\hat{\sigma}_a$ of 1.63 and 1.89 respectively) and for gill rakers ($\hat{\sigma}_a$ of 0.55 and 0.40), but not for pectoral fin rays ($\hat{\sigma}_a$ of 0.29 and 0.32). The inconsistency of the pectoral fin results with the a priori expectation of higher asymmetry in low pH situations can be seen in plots of $\hat{\sigma}_a$ against age (Figure 3-6). $\hat{\sigma}_a$ was quite variable among the different age classes in both the low pH and circumneutral lakes, resulting in no consistent difference among pH classes. For lateral line scales,

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Table 3-6. Asymmetry parameter estimates $\hat{\mu}$, $\hat{\sigma}_L$, $\hat{\sigma}_a$, and $|\bar{A}|$ for bluegills collected from eleven Wisconsin lakes, listed in order of increasing pH. Data from Weiner and Rago (1987). Superscript s indicates significant skew of the A_i distribution; k indicates significant kurtosis.

		Maximum sample	Pored Lateral line scales				Pectoral fin rays				Gill rakers on outer arch			
Lake	pH	size	$\hat{\mu}$	$\hat{\sigma}_L$	$\hat{\sigma}_a$	$\|\bar{A}\|$	$\hat{\mu}$	$\hat{\sigma}_L$	$\hat{\sigma}_a$	$\|\bar{A}\|$	$\hat{\mu}$	$\hat{\sigma}_L$	$\hat{\sigma}_a$	$\|\bar{A}\|$
Hilderbrand	5.1	33	45.1	1.8	1.05	1.16	13.3	0.2	0.08	0.12^{k}	15.8	0.6	0.59	0.67
Lumen	5.2	50	43.1	2.1	2.16	2.33^{k}	13.6	0.4	0.20	0.24^{k}	16.3	0.4	0.52	0.52^{k}
Currie	5.6	50	44.0	1.7	1.21	1.29^{k}	13.0	0.8	0.29	0.24^{sk}	15.3	0.7	0.47	0.48
Flannery	5.7	56	43.7	1.6	1.82	1.88^{k}	13.0	0.4	0.26	0.20^{sk}	15.1	0.7	0.63	0.57^{sk}
Clara	6.0	50	44.7	2.1	1.38	1.49^{sk}	13.2	0.2	0.40	0.32^{k}	15.2	0.5	0.50	0.54
Toulish	6.7	48	43.7	2.4	1.62	1.66	12.3	0.6	0.15	0.17^{k}	15.1	0.6	0.48	0.54
Hixon	6.8	51	42.6	2.3	2.55	2.54	13.4	0.2	0.11	0.20^{sk}	15.3	0.6	0.31	0.28^{sk}
Clear	6.9	50	42.6	1.3	1.56	1.65	13.1	0.3	0.21	0.18^{k}	15.1	0.3	0.38	0.46
Dorothy Dunn	6.9	16	44.0	1.4	0.83	1.00	13.3	0.3	0.36	0.19^{k}	14.7	1.1	0.32	0.38
Nelson	7.0	52	42.3	2.6	2.00	1.89^{k}	13.1	0.2	0.36	0.19^{sk}	15.6	0.5	0.33	0.33^{k}
Garth	7.5	49	43.7	1.6	1.60	1.64	13.5	0.3	0.44	0.33^{sk}	15.6	0.5	0.48	0.55

circumneutral lakes had overall a slightly higher asymmetry, while low pH lakes were overall more asymmetric than circumneutral lakes for gill rakers. The age classes, which for this study represent distinct year classes, did not show consistent asymmetry patterns across the three characters (Figure 3-6).

Interindividual variation, $\hat{\sigma}_L$, also exhibited an inconsistent relationship with pH level and age (Figure 3-7). No trend across ages was seen for gill rakers or pectoral fin rays, although gill rakers exhibited higher $\hat{\sigma}_L$ under low pH, while pectoral fin rays had higher $\hat{\sigma}_L$ at the higher pH. Lateral line scales exhibited a trend of increasing $\hat{\sigma}_L$ with increasing age, for both pH conditions, with a higher $\hat{\sigma}_L$ at circumneutral pH.

DISCUSSION

The use of the model-based maximum likelihood estimators for fluctuating asymmetry on previously collected meristic data did not lead to any startling new insights about fluctuating asymmetry or its causes. In general, the new index of asymmetry, $\hat{\sigma}_a$, demonstrated similar relationships with potential causal factors or, as did the previous analysis, no relationship. This is comforting in that the previous analyses were not substantially changed, even though the estimators used to quantify fluctuating asymmetry were potentially biased (Swain 1987).

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Figure 3-6. $\hat{\sigma}_a$ for bluegills collected from low pH and circumneutral pH Wisconsin lakes as a function of age of fish at the time of collection. Characters examined were gill rakers (GR), pectoral fin rays (PFR), and lateral line scales (LAS).

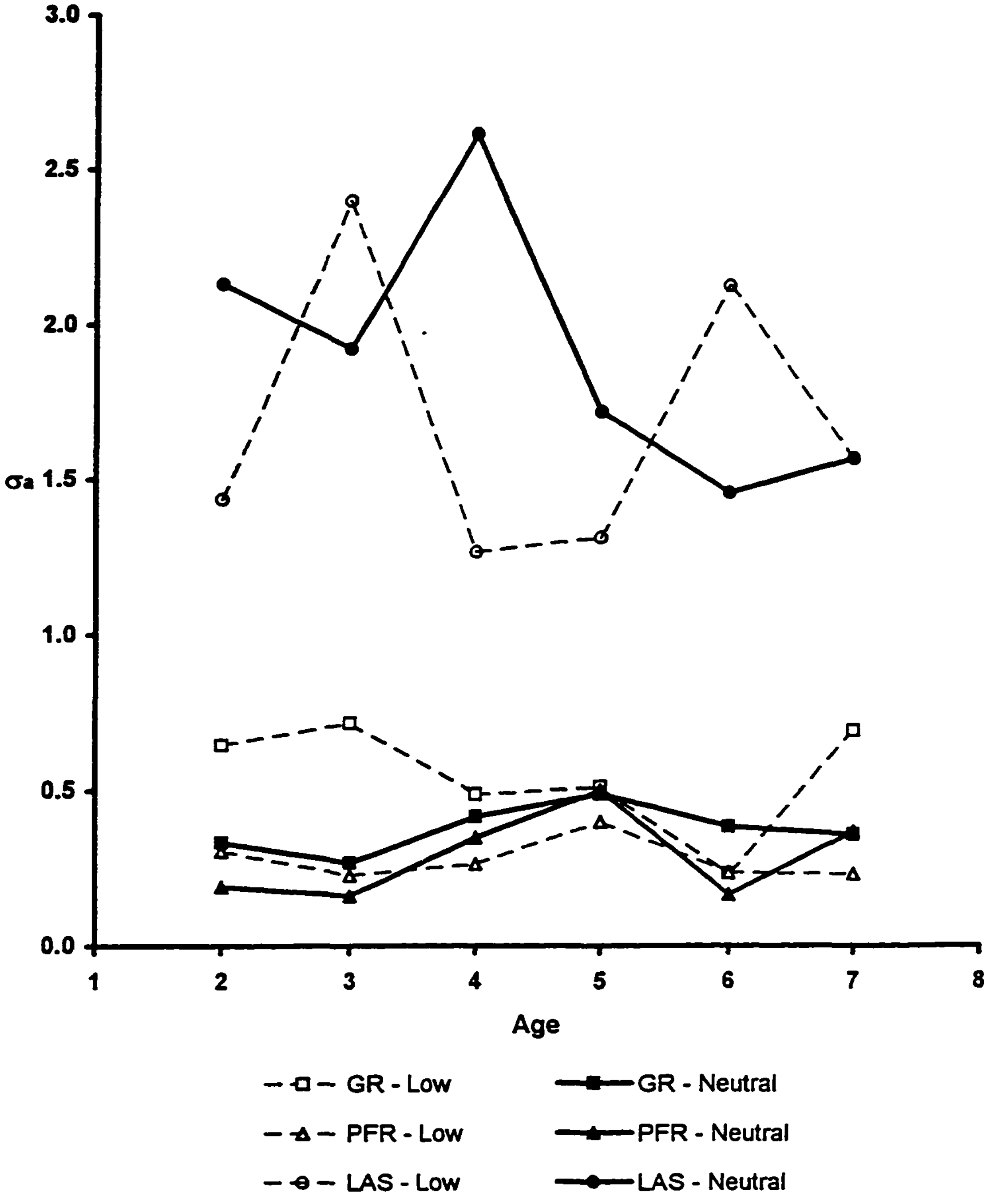

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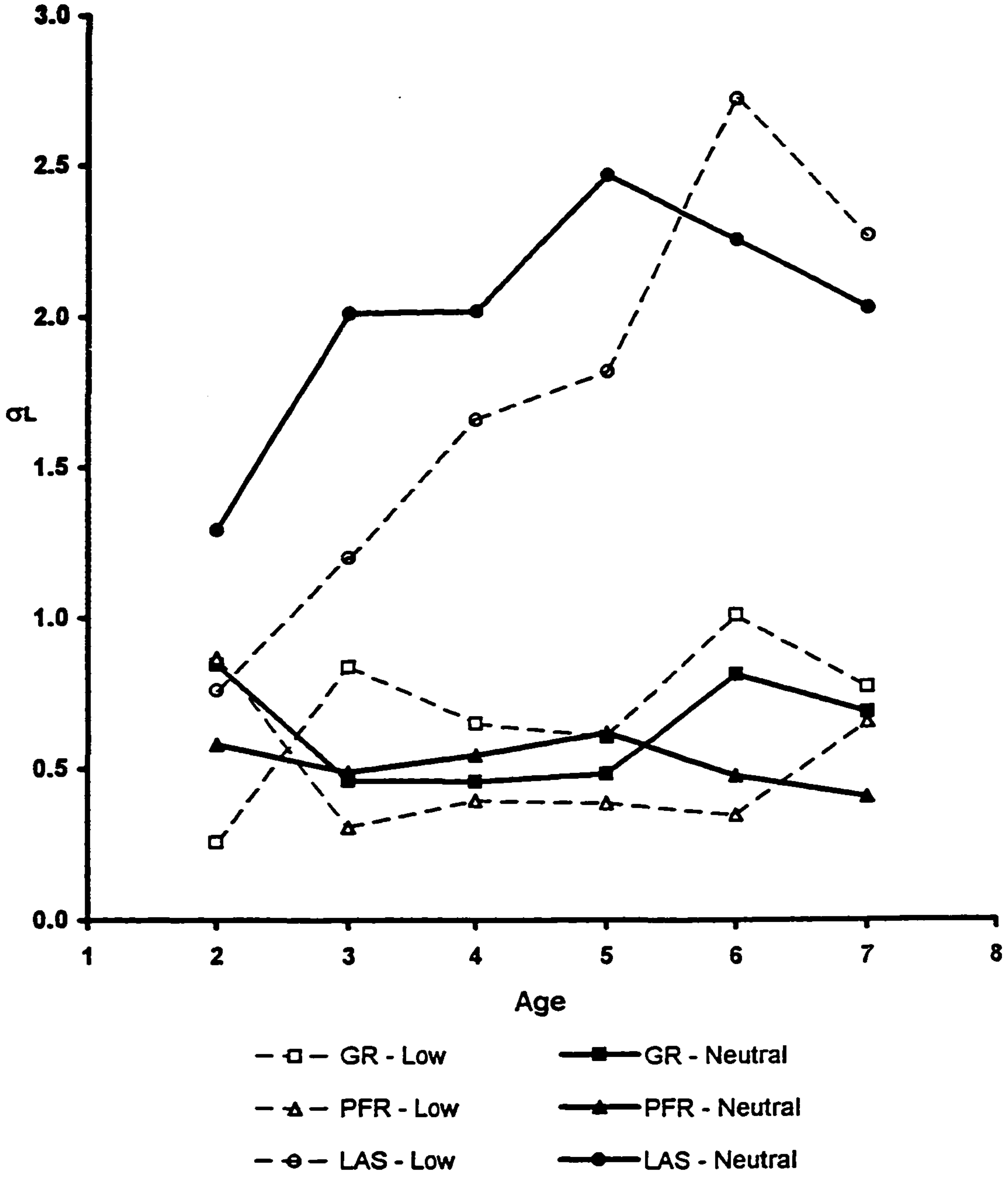
Figure 3-7. σ_L for bluegills collected from low pH and circumneutral pH Wisconsin lakes as a function of age of fish at the time of collection. Characters examined were gill rakers (GR), pectoral fin rays (PFR), and lateral line scales (LAS).



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The present methods, like those based on A_i, are based on an assumption about the distributional form of the data. The F-test, commonly used to test differences in s_A^2 among populations requires normality of the A_i. In many instances, the A_i values exhibited significant departures from normality, particularly for skewness and kurtosis. Kurtosis can significantly affect the error rates of the F-test (Markowski and Markowski 1990). Palmer and Strobeck (1992) suggested that leptokurtic distributions probably result from a mixed sample from populations with different levels of fluctuating asymmetry (Type II leptokurtosis).

The three-parameter developmental model (Chapter 2) is based on the assumption that deviations due to interindividual variation in the liability and developmental instability are both normally and independently distributed, and that the deviations are additive. I did not use any explicit tests of the assumptions deriving from the conceptual model, except to compare it to the previous two-parameter conceptual model that did not include interindividual variation in the liability. In essentially all cases, the three parameter model was significantly better than a model which did not include this parameter. Thus, the model may not be right, but it is a better descriptor of the data than the previous model.

An important advantage of the model-based estimators is the ability to partition the total character variation into components due to between-individual factors such as

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environmental conditions and genetic differences, and within-individual factors (developmental instability). The estimates of $\hat{\sigma}_L$ appear that they may also be useful in understanding the causes and ultimate uses for fluctuating asymmetry. Nevertheless, when the prediction of Soule (1979) that interindividual variation should increase with increasing heterozygosity was examined, little support was found for that hypothesis in either the rainbow trout or Sonoran topminnow data. However, the range of heterozygosity in the rainbow trout strains examined may exceed the range of values at which the hypothesis would hold true (Vrijenhoek and Lerman 1982).

When examined across populations, $\hat{\sigma}_L$ and $\hat{\sigma}_a$ appear to be positively related in some cases, which might suggest that fluctuating asymmetery is simply the result of lack of precise control of the character state on a population level, i.e. populations that have high interindividual variability will also have less control of intra-individual variability. Alternatively, factors that reduce developmental stability at the level of bilateral symmetry also can act at the level of the entire organism. These competing hypotheses cannot be resolved by comparing $\hat{\sigma}_L$ and $\hat{\sigma}_a$ among different populations from different environments. Instead, studies of a single population raised under various levels of stress, preferably a stress that would not affect μ, would be required. Although suitable experiments have been conducted with other organisms (Graham et al. 1993,

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Clarke 1992), very little experimental work has been done with fish since Valentine and Soulé (1973).

Other factors may also be acting to obscure the causes of fluctuating asymmetry in these fish studies. First, the sample sizes in nearly all of these studies are typically on the order of 50 fish or less from any population. This sample size was shown to have relatively low power, even for the likelihood ratio test for $\hat{\sigma}_a$, unless differences in asymmetry are large (Chapter 2).

Second, in many cases the sample contains a range of ages. For fish, a sample of mixed ages will contain individuals that experienced a range of conditions during the relatively short period when meristic features are established. It is relatively easy to hypothesize that the levels of developmental stress might vary widely across year classes, or even among fish of the same year class spawned at different locations or different times in the spawning season. Thus, to the extent that fluctuating asymmetry is determined by environmental factors that can vary inter or intra-annually, this may obscure fluctuating asymmetry trends related to other causes. Samples containing a mix of cohorts with different fluctuating asymmetry levels would be one explanation for the prevalence of significant kurtosis in the A_i distributions (Palmer and Strobeck 1992). In addition, several investigators have found age-related trends in fluctuating asymmetry patterns in fish (Moran 1997, Zakharov and Zyuganov 1980), with younger fish showing

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higher levels of asymmetry. This would be consistent with a reduction in survival for asymmetric individuals, which has been demonstrated in fish (Quattro and Vrijenhoek 1989) and many other organisms (See Møller 1996 for a review).

Another potential reason for inconsistent patterns of fluctuating asymmetry could be errors in character counts or data entry. Although all of the data sets I examined appeared to be free of gross errors, only Weiner and Rago (1985) indicated any repetition of counts in order to ensure accuracy. However, others may have done replicate measurements and failed to include it in their description of methods. In any case, none of these studies reported error rates or estimated the additional variance that would be due to miscounts. While I doubt that any substantial degree of error exists in any of these data sets, even a relatively small error occurring in only 5% of the samples would bias $\hat{\sigma}_a$ by approximately 10% (Chapter 2). This level of error might be enough to obscure trends. During data entry for the stickleback data, I incorrectly assigned two complete morph individuals to the low morph group. This had substantial adverse effects on the analysis.

Selection of characters for analysis can also affect the probability of success in demonstrating fluctuating asymmetry. Ideally, investigators would select characters that are not strong determinants of fitness because those characters are often highly canalized and show little variation either among or within individuals. Pectoral fin

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rays are commonly used because 1) they are easily accessible; 2) the character state is usually relatively low which allows for rapid and accurate counts; and 3) there is no strong relationship with fitness readily apparent. However, their location external to the body of the fish makes them susceptible to damage, which may not be apparent when counting rays and could thus generate incorrectly large values of A_i, which is an alternative explanation for why this character is often leptokurtotic. Pelvic fin rays are also commonly used, but this fin seems to often have too little variation to be useful for ascertaining fluctuating asymmetry.

This examination of past fluctuating asymmetry studies suggests that many previous studies have suffered from use of less than optimal statistical tests and from sample sizes that could reliably detect only large differences in fluctuating asymmetry. Bias due to shifts in the character mean may also have acted to obscure real patterns in fluctuating asymmetry, however, the essentially unbiased methods developed in Chapter 2 did not indicate that the previous analyses were substantially in error. In the future, fluctuating asymmetry studies on fish would be more likely to successfully find real relationships if they used larger samples sizes and employed analysis techniques, such as those presented here, that are not susceptible to bias from the character mean.

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REFERENCES

Ames, L. J., J. D. Felley, and M. H. Smith. 1979. Amounts of asymmetry in centrarchid fish inhabiting heated and nonheated reservoirs. Transactions of the American Fisheries Society 108:489-495.

Bell, M. A. 1982. Differentiation of adjacent stream populations of threespine sticklebacks. Evolution 36:189-199.

Carline, R. F. and M. V. Lawal. 1985. Contaminants and bilateral asymmetry in yellow perch. Environmental Toxicology and Chemistry 4:543-547.

Clarke, G. M. 1992. Fluctuating asymmetry: A technique for measuring developmental stress of genetic and environmental origin. Acta Zoologica fennica 191:31-35.

Felley, J. 1980. Analysis of morphology and asymmetry in bluegill sunfish (Lepomis macrochirus) in the southeastern United States. Copeia 1980(1), pp.18-29.

Graham, J. H. and J. D. Felley. 1985. Genomic coadaptation and developmental stability within introgressed populations of Enneacanthus gloriosus and E. obesus (Pisces, Centrarchidae). Evolution 39:104-114.

Graham, J. H., K. E. Roe, and T. B. West. 1993. Effects of lead and benzene on the developmental stability of Drosophila melanogaster. Ecotoxicology 2:185-195.

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Jagoe, C. H. and T. A. Haines. 1985. Fluctuating asymmetry in fishes inhabiting acidified and unacidified lakes. Canadian Journal of Zoology 63:130-138.

Leary, R. F., F. W. Allendorf, and K. L. Knudsen. 1992. Genetic, environmental, and developmental causes of meristic variation in rainbow trout. Acta Zoologica Fennica 191:79-95.

Markow, T. A. 1994. Developmental Instability: Its Origins and Evolutionary Implications. Kluwer Academic Publishers. Netherlands.

Markowski, C. A. and E. P. Markowski. 1990. Conditions for the effectiveness of a preliminary test of variance. The American Statistician 44:322-326.

Møller, A. P. 1997. Developmental stability and fitness: a review. The American Naturalist 149:916-932.

Moran, P, J. I. Izquierdo, A. M. Pendas, and E. Garcia-Vazquez. 1997. Fluctuating asymmetry and isozyme variation in Atlantic salmon: Relation to age of wild and hatchery fish. Transactions of the American Fisheries Society 126:194-199.

Palmer, A. R. 1994. Fluctuating asymmetry analyses: A primer. Pages 335-364 In T. A. Markow (ed.), Developmental Instability: Its Origins and Evolutionary Implications. Kluwer, Dordrecht, Netherlands.

Palmer, A. R. and C. Strobeck. 1992. Fluctuating asymmetry as a measure of developmental stability: Implications

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of non-normal distributions and power of statistical tests. Acta Zoologica Fennica 191:55-70.

Quattro, J. M. and R. C. Vrijenhoek. 1989. Fitness differences among remnant populations of the endangered Sonoran topminnow. Science 245:976-978.

Soulé, M. E. 1979. Heterozygosity and developmental stability: Another look. Evolution 33:396-401.

Swain, M. W. 1987. A problem with the use of meristic characters to estimate developmental stability. The American Naturalist 129:761-768.

Valentine, D. W. and M. Soulé. 1973. Effect of p,p'-DDT on developmental stability of pectoral fin rays in the grunion, _Leuresthes tenuis_. Fishery Bulletin 71:921-926.

Valentine, D. W. and M. E. Soulé, and P. Samallow. 1973. Asymmetry analysis in fishes: A possible statistical indicator of environmental stress. Fishery Bulletin 71:357-370.

Van Valen, L. 1962. A study of fluctuating asymmetry. Evolution 16:125-142.

Vrijenhoek, R. C. and S. Lerman. 1982. Heterozygosity and developmental stability under sexual and asexual breeding systems. Evolution 36:768-776.

Wiener, J. G. and P. J. Rago. 1987. A test of fluctuating asymmetry in bluegills (_Lepomis macrochirus_ Rafinesque) as a measure of pH-related stress. Environmental Pollution 44:27-36.

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Zakharov, V. M. and V. V. Zyuganov. 1980. Assessment of asymmetry of bilateral characters as a population characteristic. Ékologiya 1:10-16.

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CHAPTER 4

SOURCES OF VARIATION IN FLUCTUATING ASYMMETRY OF PECTORAL FIN RAY COUNTS IN STRIPED BASS (*Morone saxatilis*)

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ABSTRACT

Fluctuating asymmetry of pectoral fin rays was examined in two samples of east coast anadromous striped bass. A sample of 1,439 adult striped bass were collected from the Roanoke River, Chesapeake Bay, and Hudson River stocks in 1974-1975. An additional 370 fish were collected from these same stocks in 1989, providing a large sample of fish over many year classes.

The individual right-left differences (A) were found to frequently depart from normality, primarily by being leptokurtotic. Fluctuating asymmetry was estimated by a three-parameter developmental model, and by standard deviation of A, mean absolute A, and the proportion of individuals asymmetric. Tests based on the three-parameter model indicated significant differences among the three river system stocks, and between the two data sets for each of the three stocks. Tests based on the other three asymmetry indices were not as powerful at detecting these differences.

When the data were segregated by year class and subjected to a nested ANOVA, Year within stock and river was the only variance component that exceeded the magnitude of the error component, indicating that year-to-year variation in fluctuating asymmetry is large for these populations and could obscure any differences due to stock, or river within stock.

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INTRODUCTION

In Chapter 3, previous studies of fluctuating asymmetry in fishes were reexamined using the three-parameter asymmetry model to see whether the conclusions reached by the authors would change using the new methodology. Although the new methods appear to have more powerful tests for differences in fluctuating asymmetry among populations, the basic conclusions were not substantially changed. The relationships that were expected between fluctuating asymmetry and such factors as heterozygosity and pH of the environment were no more apparent with the new indices than they were with the various indices used by the original authors.

There could be many reasons for the failure, even using the new methods, to find clear relationships with the potential causes of developmental instability. One key reason may be that the relationships did not exist for these populations. Certainly, the relationship between fluctuating asymmetry and heterozygosity has recently been questioned (Graham 1993, Clarke 1994). Coadaptation of gene complexes is now proposed as the true cause of the genetic stress that has been seen to arise in hybrid or extremely inbred populations. Regardless of which is the true cause of developmental instability, in actuality the two factors usually vary together so that it is difficult to separate their effects.

For contaminant stress, results of studies of wild fish

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have been very inconsistent. Although Jagoe and Haines (1985) found some increase in fluctuating asymmetry with decreasing pH for one of the three species they studied, Weiner and Rago (1987) did not. However, neither of those studies included experiments which would determine the sensitivity of meristic characters to low pH, nor did they measure the actual pH to which their specimens were exposed during development of their meristic characters. This shortcoming would not be a problem if experimental results were available to demonstrate that pH actually affects the developmental stability of the characters examined, and if there was more information about the within and among year variation in pH in the lakes under study. (Indeed, the information on pH variability may be available, however it was not presented in the studies).

Even if the hypothesized relationships between developmental stability and heterozygosity or pH are real, there are several additional reasons why these relationships could be difficult to demonstrate. First, in all of these studies (except for Bell's data on threespine stickleback), the sample size is approximately 50 or less. Although Graham et al. (1993) suggested that samples should be at least 40, the ability to detect differences among populations with this sample size is not very great unless the differences are large (Chapter 1).

In most of these studies, the samples represented a mixture of annual cohorts, which could have experienced

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differing levels of stress during development. Even if the stressor of interest, e.g. pH, did not vary much during the developmental period between years, other factors such as temperature may have, and could result in year to year differences in developmental stability.

A second potential confounding factor is errors in counts of the meristic characters. Although there is no reason to suspect that any of these studies is seriously compromised by this type of error, only one of them (Weiner and Rago 1987) indicated that the characters were recounted. [Jagoe and Haines (1985) conducted replicate counts on some of the brook trout (Salvelinus fontinalis) in their study.] All of the indices of fluctuating asymmetry examined were relatively sensitive to counting errors, thus even a small amount of counting error could bias the estimates.

A third difficulty in interpretation of many of the field studies is that differences in the populations' genetic heterozygosity is usually confounded with different environments. Thus differences in level of developmental instability cannot be clearly attributed to either genetic or environmental factors.

In this chapter, the variability of several of the common indices of fluctuating asymmetry will be examined with respect to inter-annual variation in the environment. The data used for this analysis are pectoral fin ray counts of anadromous East coast striped bass (Morone saxatilis) collected in 1974-1975 and 1989 during two separate stock

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discrimination studies (Grove et al.1976, Waldman et al.1997). The advantage of these striped bass data is that they overcome several of the key difficulties in the previously published data. First, the overall sample size is quite large, and samples from some annual cohorts are also large in comparison to most published studies. Second, all of the fish in the 1974-1975 data set were recounted and differences in the counts were resolved, thus eliminating counting errors as a source of error in the estimates.

Finally, east coast striped bass stocks are genetically monomorphic. Both protein surveys (Otto 1975; Grove et al. 1976; Siddell et al. 1980) and mitochondrial DNA (Chapman 1990; Wirgin et al. 1990) surveys have shown extreme monomorphism within and among striped bass populations. If heterozygosity provides a buffer against developmental instability as suggested by some workers (Palmer and Strobeck 1986; Parsons 1990), then striped bass should be especially sensitive to stresses that induce fluctuating asymmetry because of the low heterozygosity noted for this species. Differences in fluctuating asymmetry between stocks are more likely the result of different environmental stresses in the native rivers than to differences in heterozygosity.

METHODS

Asymmetry in pectoral fin ray counts was evaluated in 1,439 striped bass collected during the spring spawning period from three principal east coast striped bass stocks,

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i.e., Hudson River, Chesapeake Bay, and Roanoke River during 1974-1975 (Grove et al. 1976). Sample sizes were 364 from the Hudson River, 976 from Chesapeake Bay, and 99 from the Roanoke River (Table 4-1). These fish originated from the 1958-1973 year classes, but the 1964-1972 year classes were most prevalent. The Chesapeake Bay sample comprised 306 fish from the Elk River (Upper Bay), 254 from the Potomac River, 191 from the Choptank River, and 225 from the Rappahannock River.

In the spring of 1989, pectoral fin ray counts were also made on 370 striped bass collected for a stock identification study (Waldman et al. 1997) from the same rivers as the 1974-1975 samples. Of the 370 fish, 133 were from the Hudson River, 191 were from the Chesapeake Bay, and 46 were from the Roanoke River (Table 4-2). The 1989 sample contained fish from the 1979-1987 year classes, although the 1983-1987 year classes were most prevalent. The 1989 Chesapeake Bay sample was made up of 47 fish from the Chesapeake and Delaware Canal (Upper Bay), 51 from the Potomac River, 48 from the Choptank River, and 45 from the Rappahannock River. For both surveys, pectoral fin rays were enumerated by the criteria of Hubbs and Lagler (1947) and differences between right and left side counts ($A_i = Y_{ir} - Y_{il}$) were calculated for each fish.

The distributions of the A_i values of pectoral fin ray counts were examined for departures from normality (Palmer 1994) by testing for mean $A_i = 0$, and for skewness (g_1) and

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Table 4-1 Sample size of striped bass collected from east coast estuaries during 1974-1975 stock discrimination studies.

Year Class	Roanoke	Rappa- hannock	Potomac	Choptank	Elk/Upper Bay	Hudson
1958	0	1	3	2	0	2
1959	0	0	1	0	0	1
1960	0	0	3	2	0	1
1961	0	0	1	2	0	0
1962	0	0	4	0	0	9
1963	0	1	6	2	2	30
1964	2	1	23	3	12	65
1965	0	4	16	4	8	72
1966	3	21	23	9	8	62
1967	2	5	19	7	7	20
1968	4	7	6	9	4	15
1969	7	37	23	24	62	41
1970	16	130	88	57	165	26
1971	32	9	15	45	27	11
1972	25	2	6	20	6	0
1973	7	3	0	0	0	0
Unknown	1	4	17	4	5	9
Total	99	234	254	190	306	364

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Table 4-2 Sample size of striped bass collected from east coast estuaries during 1989 stock discrimination studies.

Year Class	Roanoke	Rappa-hannock	Potomac	Choptank	Elk/Upper Bay	Hudson
1981	0	0	1	1	1	1
1982	0	1	3	1	2	8
1983	0	0	3	3	3	19
1984	0	2	4	3	3	37
1985	2	5	17	4	5	7
1986	6	11	21	6	31	15
1987	33	24	1	24	2	37
Unknown	5	2	1	6	0	4
Total	46	45	51	48	47	130

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kurtosis (g_2) with a t-test (Sokal and Rohlf 1995).

The indices of fluctuating asymmetry were $|\overline{A}|$, s_A, $\hat{\rho}$, and the maximum likelihood estimates for the three parameter model, ($\hat{\mu}$, $\hat{\sigma}_L$, $\hat{\sigma}_a$) calculated from a gradient search algorithm (Chapter 2). Tests for asymmetry differences among stocks were done with a Kruskal-Wallis, F-test, G-test, and generalized likelihood ratio test. Fluctuating asymmetry differences among stocks along a latitudinal gradient were examined by comparing the Hudson River to Chesapeake Bay stocks, and Chesapeake Bay to Roanoke River stocks.

The pectoral fin asymmetry data are naturally divided into three stocks; in addition, data for the Chesapeake system can be further divided into four groups corresponding to the four spawning rivers sampled. Cohort data for each system are available for several years. This structure suggested that a Model II (random effects) nested ANOVA (Sokal and Rohlf 1995) would be appropriate to estimate the relative influence of stock, river within stock, and year within river and stock effects on asymmetry.

To reduce variance heterogeneity in part caused by differences in sample size (Table 4-1) and to provide replicates for estimation of SS_{error}, an approach developed by Scheffe' (1959) and recommended for use in asymmetry studies (Palmer and Strobeck 1986) was used. River-year combinations with less than 20 fish were eliminated; those with 40 fish or more were randomly divided into subsets

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containing at least 20 fish. The asymmetry indices were calculated for each subset, so that river-year combinations with large sample sizes had multiple estimates of the asymmetry. Of the 1809 fish collected, 1325 were included in the ANOVA. The results were examined for the relative importance of the model components, rather than statistical significance of the factors (Sokal and Rohlf 1969; Matthews 1990; Toft 1990). The ANOVA was conducted using the General Linear Model procedure from the Statistical Analysis System (SAS 1987).

To investigate the importance of environmental factors that may influence asymmetry, asymmetry ($\hat{\sigma}_a$) for the nine year classes from the Hudson River with a sample size of at least 20 were regressed against flow and temperature data. For May and June, which is the time when larval development occurs in striped bass, daily flow and temperature records were obtained from the USGS Green Island monitoring station and the City of Poughkeepsie water intake, respectively. For the first and second half of each month, the mean temperature and flow were calculated for each year. Additionally, an index of instability of environmental conditions was created from the maximum absolute change in flow and temperature over two days within each semimonthly period. Multiple regression models were determined for one, two, and three variable models using forward selection methods in the Regression procedure in SAS. Sample size (number of fish used to calculate

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asymmetry) was used to weight the observations.

RESULTS

Pectoral fin ray counts of striped bass are a moderate-sized meristic character, typically ranging between 15 and 19, with a mean near 16.5. The distribution of the A_i values for pectoral fins was decidely non-normal. The individual asymmetry values were significantly skewed for 9 of the 12 data sets and leptokurtotic in all 12 data sets (Figure 4-1). Despite significant skewness, mean asymmetry values ranged from only -0.131 to 0.255, and were not significantly different from zero, thus no directional asymmetry is apparent. The unimodal concentration at a value of zero indicates that pectoral fin ray counts are typically symmetric.

The asymmetry statistics generated from the 3-parameter model ($\hat{\mu}$, $\hat{\sigma}_L$, $\hat{\sigma}_a$) indicated significant differences in fluctuating asymmetry among the three stocks, and between the two data sets for all three stocks (Table 4-3). $\hat{\sigma}_a$ ranged from 0.647 for the Chesapeake Bay stock, to 0.737 for the Roanoke stock. The Chesapeake stock was significantly different from both the Hudson and Roanoke stocks. However, the overall view of stock differences is somewhat confounded by comparisons of the two periods within each stock. The Hudson stock exhibited a significant increase in $\hat{\sigma}_a$ from the 1974-75 data to 1989, while the Chesapeake Bay and Roanoke stocks had significant declines

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in $\hat{\sigma}_a$.

Although no tests for significance were conducted, the values of $\hat{\sigma}_L$ appear to be much less variable than $\hat{\sigma}_a$, both within and among stocks. The range of $\hat{\sigma}_L$ among the stocks was only 0.363 (Roanoke) to 0.438 (Chesapeake Bay), and the differences in $\hat{\sigma}_L$ between data sets within a stock were only 0.1 for the Roanoke stock and approximately 0.05 f or the Hudson River and Chesapeake Bay.

Fluctuating asymmetry estimates based on $\hat{p}$ and $|\bar{A}|$ produced similar qualitative patterns and significant differences among and within stocks. Both indices gave similar levels of fluctuating asymmetry for the Hudson and Chesapeake Bay stocks overall, with significantly lower fluctuating asymmetry for the Roanoke stock. Within stock differences were significant only for the Chesapeake Bay stock, in which a decline in fluctuating asymmetry is evident in the 1989 data set.

s_A indicated no significant diffences in the overall levels of fluctuating asymmetry among stocks, with s_A varying only from 0.968 (Chesapeake Bay) to 1.067 (Roanoke). Within stocks, the Hudson exhibited a significant increase in fluctuating asymmetry from the 1974-75 data to the 1989 data, while the Roanoke stock had a significant decline.

In contrast to the variability of the inter- and intra-stock comparisons for the different fluctuating asymmetry indices, the nested ANOVA results were relatively consistent among the four estimators (Table 4-4). For all

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Figure 4-1 Distributions and statistics for A_i for pectoral fin rays of east coast striped bass sampled in 1974-1975 and 1989. Mean $A_i = 0$, skewness (g_1), and kurtosis (g_2) were tested for significance using t-tests. * indicates $p < 0.05$; ** indicates $p < 0.01$.

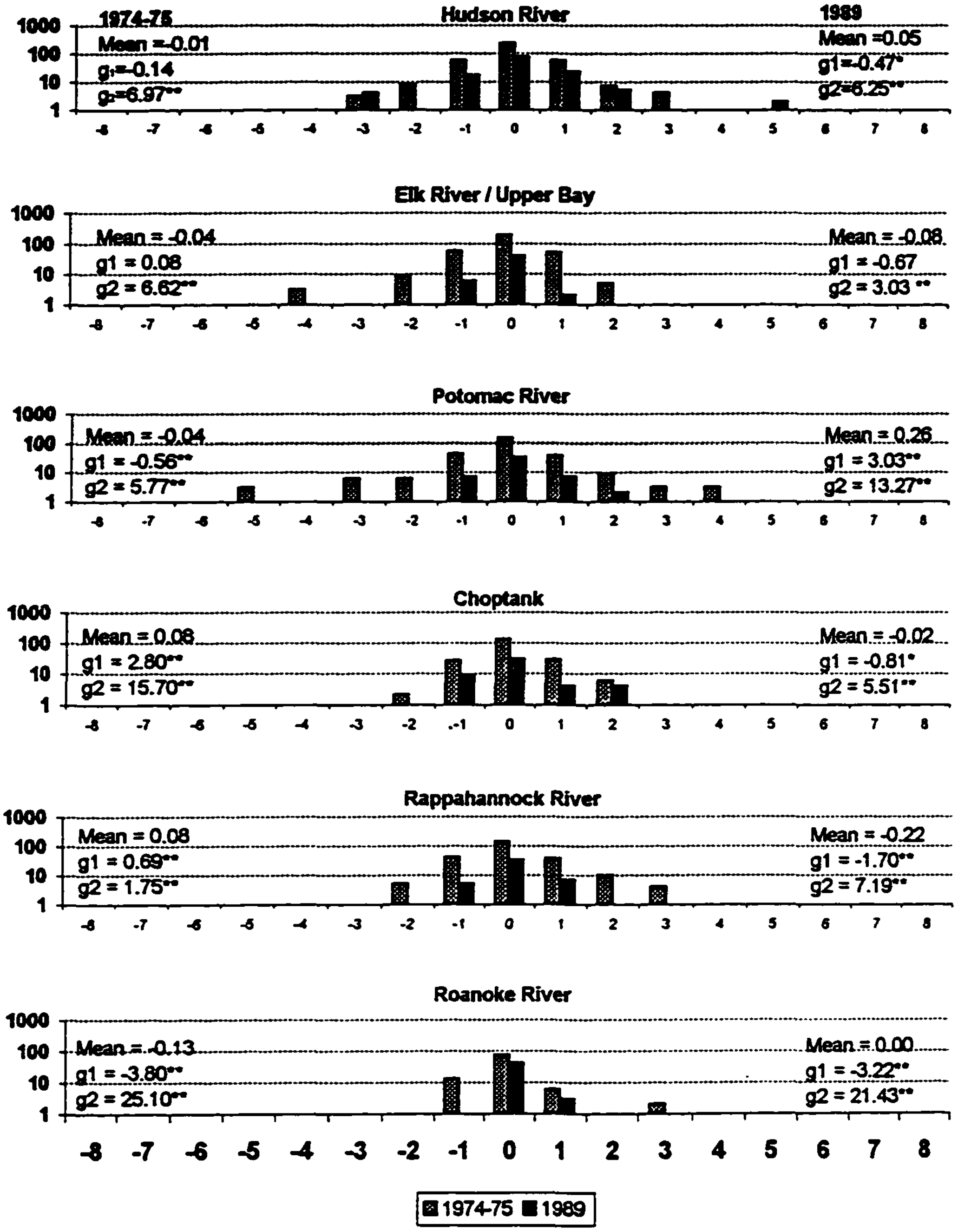

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Table 4-3 Asymmetry statistics for pectoral fin rays from east coast striped bass stocks. Different exponents for the asymmetry indices indicate significant ($p < 0.05$) differences. Lower case used for within stock comparisons; upper case for among stock comparisons.

Stock	Data Set	NLL	$\hat{\mu}$	$\hat{\sigma}_L$	$\hat{\sigma}_a$	s_A	$\lvert\bar{A}\rvert$	$\hat{p}$
Hudson	1974-75	817.65	16.50	0.387	0.594^a	0.906^a	0.481	0.371
	1989	384.66	16.36	0.425	0.946^b	1.381^b	0.723	0.431
	Combined	1233.74	16.47	0.401	0.710^A	1.052	0.545	0.387
Chesapeake	1974-75	2384.12	16.54	0.433	0.662^a	0.988	0.537^a	0.403^a
	1989	413.28	16.82	0.380	0.567^b	0.859	0.393^b	0.298^b
	Combined	2817.12	16.58	0.438	0.647^B	0.968	0.513^B	0.386^B
Roanoke	1974-75	258.42	16.53	0.256	0.827^a	1.193^a	0.414	0.242
	1989	87.12	16.04	0.356	0.488^b	0.722^b	0.217	0.130
	Combined	362.23	16.37	0.363	0.737^C	1.067	0.352^C	0.207^C

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Table 4-4 Results of nested analysis of variance on fluctuating asymmetry indices. MS indicates the means square for the source, Var is the estimated variance component due to the source.

		δ_L		δ_a		s_A		$\lvert\bar{A}\rvert$		$\hat{\beta}$	
Source	df	MS	Var	MS	Var	MS	Var	MS	Var	MS	Var
Stock	3	0.015	-0.003	0.065	0.004	0.073	0.007	0.043	0.004	0.073	0.005
River(Stock)	3	0.054	0.001	0.083	-0.015	0.129	-0.031	0.057	-0.015	0.009	-0.002
Year(Stock,River)	24	0.035	0.003	0.122	0.052	0.222	0.103	0.104	0.051	0.018	0.003
Error	26	0.030	0.030	0.038	0.038	0.056	0.056	0.022	0.022	0.013	0.013
R^2		0.5702		0.7694		0.8000		0.8302		0.6461	
Model p		0.3292		0.0030		0.0008		0.0001		0.0001	

Note: Variance components were estimated from the equations for expected mean squares.

$$EMS_{Stock} = Var_{Error} + 1.9416\ Var_{Year(Stock,River)} + 8.7983\ Var_{River(Stock)} + 13.411\ Var_{Stock}$$

$$EMS_{River(Stock)} = Var_{Error} + 3.4184\ Var_{Year(Stock,River)} + 9.0631\ Var_{River(Stock)}$$

$$EMS_{Year(Stock,\ River)} = Var_{Error} + 1.6073\ Var_{Year(Stock,River)}$$

$$EMS_{Error} = Var_{Error}$$

four estimates of fluctuating asymmetry ($\hat{\sigma}_a$, s_A, $|\bar{A}|$, $\hat{\rho}$) the overal model was signficant , and the only source term that even approached the magnitude of the error variance was Year(within Stock,River). This term ranged from about 0.25 times the error variance ($\hat{\rho}$) to 2.4 times the error variance ($|\bar{A}|$). The Stock component was always less than 1/2 the magnitude of the error variance while all estimates of the River(within Stock) components were negative, indicating essentially no contribution from this source. These results indicate that year-to-year variation in the asymmetry estimates is large when compared to the variation among stocks or among rivers within a stock.

The ANOVA results for $\hat{\sigma}_L$ contrasted sharply with those for the other parameters. For $\hat{\sigma}_L$, the model was not significant, and none of the individual component estimates were more than 10 percent of the error component.

Multiple regression results indicated a significant annual trend of increasing fluctuating asymmetry as year was the first variable selected by the regression procedure (Table 4-5). The 1-variable model was significant, even though year was not. R^2 for the model was 0.3589. At the 2-variable stage, mean flow during the second half of May entered the model, resulting in an R^2 of 0.6791 with a significant model and both factors significant. The coefficient for freshwater inflow was negative, indicating an inverse relationship with asymmetry. At the 3-variable stage, mean temperature during the second half of June

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entered the model, resulting in a model R^2 of 0.7833. The model was significant, as were year and mean freshwater inflow. Due to the low number of years available, higher order models were not explored.

DISCUSSION

Pectoral fin rays would appear to be a good character for examining fluctuating asymmetry in striped bass. The number of fin rays is large enough (~16), and since the character is not highly canalized there is a moderate amount of inherent variability aside from that due to developmental instability. In addition, pectoral fins are externally located and their rays can be easily and unambiguously enumerated without the need for complex and expensive laboratory techniques. Although pectoral fins undergo a longer ontogeny than some other fins (Vladykov 1934), they are still formed within a short interval of larval life (Kendall et al. 1984), and thus may reflect conditions within highly constrained temporal and geographic limits.

Distributions of the within-individual differences (A_i), for samples from individual spawning rivers were significantly non-normal; all distributions were leptokurtotic and most were also significantly skewed, while none exhibited directional asymmetry. Leptokurtosis often indicates that the sample comes not from a single population (with respect to the level of developmental instability),

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Table 4-5 Results of weighted multiple regression of δ_a on environmental variables for Hudson River striped bass stock for 9 annual cohorts with sample size ≥ 20. Sample sizes used for weighting. * indicates factor $p < 0.05$.

	Variables in Model		
	1	2	3
Model SS	0.2262	0.4279	0.4935
Error SS	0.4039	0.2022	0.1366
r^2	0.3589	0.6791	0.7833
Model p	0.0882	0.0330	0.0409
Factors and Coefficients			
Year	0.0191	0.0203*	0.0306*
Mean Flow in Late May		-0.00002*	-0.00002*
Mean Temp in Late June			-0.1694

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but from several populations with differing levels of instability (Palmer and Strobeck 1992). This seems a reasonable explanation for the striped bass data since each of the river samples was composed of a mix of year classes, each of which was subject to a different set of environmental conditions during its early developmental stages. Although we don't yet know how the set of environmental conditions in the natal estuary affect asymmetry, the inherent variability of estuaries prior to and during the striped bass spawning season, coupled with variable inputs and availability of contaminants, it seems reasonable to expect that fluctuating asymmetry could vary among year class for this species. The multiple regression conducted on the Hudson River data suggests that freshwater inflow in late May, or some consequence of freshwater inflow, may contribute to the asymmetry level of the cohort; larger flows, through an unknown mechanism, tended to produce year classes with lower asymmetry levels.

The year-to-year variability observed for striped bass has important implications for the further study of fluctuating asymmetry in this species or in other species with similar life histories. For striped bass it is critical to be able to accurately determine when individuals were spawned in order to segregate the sample into year-specific cohorts. Even so, it could be that annual cohorts still are subject to a large amount of variability in the conditions during early development. Ulanowicz and Polgar

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(1980) suggested that most striped bass larvae in the Chesapeake Bay system end up in environments that are not suitable for survival. If spatial and temporal variability of environmental conditions within a spawning river is large enough to affect survival, then certainly effects on developmental stability would not seem unlikely.

The ways in which environmental variability will affect developmental stability are not sufficiently known. Many environmental factors are potential sources of sublethal stress in the highly variable estuarine environment in which striped bass larvae develop. Fluctuations in temperature (Dey 1981; Uphoff 1989), salinity (Morgan et al. 1981), freshwater flow (Summers and Rose 1987; Uphoff 1989), and pH (Hall 1987), and interactions among these variables, can all influence year class success. Some of the factors (temperature, oxygen, salinity) are also known to influence development of meristic characters in fish (Blaxter 1984) These factors may individually have direct effects on developmental stability, or they may interact with each other to affect developmental stability.

Chemical contamination has also been implicated as a possible contributing factor to the decline of east coast stocks of striped bass (Goodyear 1985; Hall 1991) Various striped bass life stages are susceptible to lethal effects of contaminant mixtures that occur in east coast rivers (Finger and Bulak 1988). Mehrle et al. (1982) found that Hudson River striped bass had higher PCB and cadmium

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concentrations and weaker vertebrae than Potomac River fish. Buckley et al. (1985) observed a correlation between a suite of biochemical indicators of sublethal stress on juvenile striped bass and previous findings of higher body burdens of contaminants in Hudson River striped bass. These correlations were significant for Hudson River fish, but not Potomac River fish (Buckley et al. 1985). Furthermore, if contaminants do stress developing larvae, it is not clear that contaminant exposure occurs only from ambient conditions. Maternal transmission of lipid-soluble contaminants may occur through the eggs (Westin et al. 1985). The contaminant concentration passed on to the gametes from the parents might also vary with age of the parents or in an age-independent fashion from one year to the next. Older striped bass are known to produce eggs with larger yolks and oil globules (Eldridge et al. 1979) which is where contaminants such as PCBs would accumulate

Environmental conditions may also mediate or amplify the effects of contamination. For instance, high inflow events could bring upstream contaminants, such as PCB-laden sediments, into the spawning area, or temperature may affect contaminant fluxes from sediments to the water.

The high year-to-year variability, and relatively large measurement error for fluctuating asymmmetry (based on the samples sizes of 20-40 individuals) argues for much larger sample size for fluctuating asymmetry studies than have typically been used. The measurement error associated with

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samples sizes less than fifty makes it difficult to detect differences among samples unless the differences are quite large. Even with large samples, variation in fluctuating asymmetry among cohorts within a natal stream will make comparisons across natal streams difficult. Although this study examined over 1800 fish, samples were sufficient to estimate fluctuating asymmetry levels precisely for only a few annual cohorts.

An additional factor that must be considered in studies of long-lived fish is selection against asymmetric individuals. When multiple cohorts are sampled at a single point in time, the cohorts vary not only in the year they were hatched, but also in age at time of examination. If there is selection against asymmetric individuals, which has been observed in other fish (Moran et al. 1997), then asymmetry of a cohort will decline as the cohort ages, and a false increasing trend in asymmetry will be suggested by the cohort data. To examine whether selection is a problem for this species, samples should be taken of early juvenile fish, soon after the fin rays have fully developed. Fluctuating asymmetry levels in these samples could be compared to additional samples of the cohort taken later in the first year of life, or at a later time. An annual series of cohorts measured just after fin development is completed would be very useful in determining how environmental conditions affect fluctuating asymmetry.

Inasmuch as both meristic counts and a large component

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of year-class strength (Uphoff 1989; Rutherford and Houde 1995) are determined during the larval stage, the environmental factors that affect symmetry may also influence year class strength. Are good year classes composed of cohorts exhibiting low levels of fluctuating asymmetry, i.e., conditions suitable for high survival generate less sublethal stress? Or, are good year classes made up of fish with a high degree of fluctuating asymmetry, indicating that conditions conducive for higher survival allow greater survival of sublethally stressed individuals? Although measures of year class strength are available for some of the rivers in this study, there were too few years with large pectoral fin samples and corresponding year class strength measures to be able to make any meaningful comparisons.

Comparison of results from the four different asymmetry measures suggests that the three-parameter model is the best way to measure asymmetry for pectoral fin rays in this species. The likelihood ratio tests for $\hat{\sigma}_a$ were able to detect differences among data sets that were not apparent using other indices and tests. However, both s_A and $|\bar{A}|$ indicated qualitatively similar patterns in asymmetry as those seen in $\hat{\sigma}_a$. The proportion of asymmetric individuals in the sample $\hat{p}$ produced different patterns of asymmetry due to its nonlinear relationship with the other indices.

Although $\hat{\sigma}_a$, if based on a sufficiently large sample size, would appear to be a useful index of developmental

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stress in striped bass, the source of that stress is not obvious. Contaminant stress might appear to be the most likely source, but there are other potential stresses for this species. The large year-to-year differences in the level of fluctuating asymmetry suggests that stress for striped bass, and perhaps other estuarine spawners, may be related to natural environmental conditions such as freshwater inflow or temperature, or to other factors controlled by these variables. A true understanding of the levels of developmental stress experienced by striped bass populations will require much more intensive sampling of fish from the wild, and also experimental studies to determine whether some of the hypothesized stresses do in fact affect developmental stability.

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REFERENCES

Blaxter, J. H. 5. 1984. Ontogeny, systematics and fisheries. American Society of Ichthyologists and Herpetologists Special Publication 1:1-6.

Boreman, J., and R. J. Klauda. 1988. Distributions of early life stages of striped bass in the Hudson River estuary, 1974-1979. American Fisheries Society Monograph 4:53-58.

Buckley, L. J., T. A. Halavik, G. C. Laurence, S. J. Hamilton, and P. Yevich. 1985. Comparative swimming stamina, biochemical composition, backbone mechanical properties, and histopathology of juvenile striped bass from rivers and hatcheries of the eastern United States. Transactions of the American Fisheries Society 114:114-124.

Chapman, R. W. 1990. Mitochondrial DNA analysis of striped bass populations in Chesapeake Bay Copeia 1990:355-366.

Clarke, G. M. 1994. The genetic basis of developmental stability. I. Relationships between stability, heterozygosity and genomic coadaptation. Pages 17-25 In T. A. Markow (ed.) Developmental Instability: Its Origins and Evolutionary Implications. Kluwer Academic Publishers. Netherlands.

Dey, W. P. 1981. Mortality and growth of young-of-the-year striped bass in the Hudson River estuary. Transactions of the American Fisheries Society 110:151-157.

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Eldridge, M. B., J. Whipple, and D. Eng. 1979. Endogenous energy sources as factors affecting mortality a nd development in striped bass (Morone saxatilis) eggs and larvae. In Early Life History of fish, Volume II. K. Sherman and R. Lasker (eds.) Springer-Verlag.

Finger, S. E., and J. S. Bulak. 1988. Toxicity of water from three South Carolina rivers to larval striped bass. Transactions of the American Fisheries Society 117:521-528.

Goodyear, C. P. 1985. Toxic materials, fishing, and environmental variation: simulated effects on striped bass population trends. Transactions of the American Fisheries Society 114:107-113.

Graham, J. H., D. C. Freeman, and J. M. Emlen. 1993. Developmental Stability: A sensitive Indicator of Populations Under Stress. Pages 136-158 in W. G. Landis, J. S. Hughes, and M. A. Lewis, editors. Environemental Toxicology and Risk Assessment. American Society for Testeing and Materials, Philadelphia.

Grove, T. L., T. J. Berggren, and D. A. Powers. 1976. The use of innate tags to segregate spawning stocks of striped bass, _Morone saxatilis_. Pages 166-176 _in_ M. Wiley, editor. Estuarine Processes. Academic Press, New York.

Hall, L. W. 1987. Acidification effects on larval striped bass, _Morone saxatilis_, in Chesapeake Bay tributaries-a

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review. Water, Air and Soil Pollution 35:87-96.

Hall, L. W., Jr. 1991. A synthesis of water quality and contaminants data on early life stages of striped bass, Morone saxatilis. Reviews in Aquatic Sciences 4:261-288.

Hubbs, C. L., and K. F. Lagler. 1947. Fishes of the Great Lakes Region. Bulletin of the Cranbrook Institute of Science 26.

Jagoe, C. H. and T. A. Haines. 1985. Fluctuating asymmetry in fishes inhabiting acidified and unacidified lakes. Canadian Journal of Zoology 63:130-138.

Kendall, A. W., Jr., E. H. Ahlstrom, and H. G. Moser. 1984. Early life history stages of fish and their characters. American society of Ichthyologists and Herpetologists Special Publication 1:11-22.

Leary, R. F., and F. W. Allendorf. 1989. Fluctuating asymmetry as an indicator of stress in conservation biology. Trends in Ecology and Evolution 4:214-217.

Matthews, W. J. 1990. Spatial and temporal variation in fishes of riffle habitats: a comparison of analytical approaches for the Roanoke River. American Midland Naturalist 124:31-45.

Mehrle, P. M., T. A. Haines, S. Hamilton, J. L. Ludke, F. L. Mayer, and M. A. Ribick. 1982. Relationship between body contaminants and bone development in east-coast striped bass. Transactions of the American Fisheries Society 111:231-241.

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Morgan, R. P., II, V. J. Rasin, Jr., and R. L. Copp. 1981. Temperature and salinity effects on development of striped bass eggs and larvae. Transactions of the American Fisheries Society 110:95-99.

Otto, R. 5. 1975. Isoenzyme sytems of the striped bass (Morone saxatilis) and congeneric percichthyid fishes. Doctoral dissertation. University of Maine, Orono.

Palmer, A. R., and C. Strobeck. 1986. Fluctuating asymmetry: measurement, analysis, patterns. Annual Review of Ecology and Systematics 17:391-421.

Parsons, P. A. 1990. Fluctuating asymmetry: an epigentic measure of stress. Biological Review 65:131-145.

Rutherford, E. S., and E. D. Houde. 1995. The influence of temperature on cohort-specific growth, survival, and recruitment of striped bass, Morone saxatilis, larvae in Chesapeake Bay. U.S. National Marine Fisheries Service Fishery Bulletin 93:315-332.

SAS. 1987. SAS/STAT guide for personal computer. SAS Institute, Cary, North Carolina.

Scheffe', H. 1959. The analysis of variance. John Wiley & Sons, New York.

Sidell, B. D., R. G. Otto, D. A. Powers, M. Karweit, and J. Smith. 1980. Apparent genetic homogeneity of spawning striped bass in the Upper Chesapeake Bay. Transactions of the American Fisheries Society 109:99- 107.

Sokal, R., and J. Rohlf. 1995. Biometry: the Principles and Practice of Statistics in Biological Research.

Reproduced with permission of the copyright owner. Further reproduction prohibited without permission.

Third Edition W. H. Freeman Company, New York.

Summers, J. K., and K. A. Rose. 1987. The role of interactions among environmental conditions in controlling historical fisheries variability. Estuaries 10:255-266.

Toft, C. A. 1990. Reply to Seaman and Jaeger: an appeal to common sense. Herpetologica 46:357-361.

Ulanowicz, R. E., and T. T. Polgar. 1980. Influences of anadromous spawning behavior and optimal environmental conditions upon striped bass (Morone saxatilis) year-class success. Canadian Journal of Fisheries and Aquatic Sciences 37:143-153.

Uphoff, J. H., Jr. 1989. Environmental effects on survival of eggs, larvae, and juveniles of striped bass in the Choptank River, Maryland. Transactions of the American Fisheries Society 118:251-263.

Vladykov, V. D. 1934. Environment and taxonomic characters of fishes. Transactions of the Royal Canadian Institute 20:99-140.

Waldman, J. R., R. A. Richards, W. B. Schill. I. Wirgin, and M. C. Fabrizio. (1997) An empirical comparison of stock identification techniques applied to striped bass Morone saxatilis. Transactions of the American Fisheries Society. 126:369-385.

Wiener, J. G. and P. J. Rago. 1987. A test of fluctuating asymmetry in bluegills (Lepomis macrochirus Rafinesque) as a measure of pH-related stress. Environmental

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Pollution 44:27-36.

Westin, D. T., C. E. Olney, and B. A. Rogers. 1985. Effects of parental and dietary organochlorines on survival and body burdens of striped bass larvae. Transactions of the American Fisheries Society 114:125-136.

Wirgin, I. I., P. Silverstein, and J. Grossfield. 1990. Restriction endonuclease analysis of striped bass mitochondrial DNA: the Atlantic coastal migratory stock. American Fisheries Society Symposium 7:475-491.

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CHAPTER 5

SUMMARY OF THE RESEARCH WITH RECOMMENDATIONS FOR FURTHER DEVELOPMENT OF THE THREE-PARAMETER MODEL

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SUMMARY OF THE RESEARCH

A three-parameter developmental model for bilateral meristic characters has been proposed and the model has been shown to fit the data for many characters in fishes much better than the existing two-parameter model. In addition to describing the model, a gradient search algorithm based on maximum likelihood principles has been constructed and proven to produce accurate parameter estimates from simulated data.

The model and fitting algorithm were examined for unbiasedness in situations which have been problematic for all previous estimators of fluctuating asymmetry of meristic characters. Of particular concern has been situations in which there is a relatively low amount of variability in a character, resulting in fluctuating asymmetry indices being sensitive to the character mean. The three-parameter model is far less sensitive to this bias than any of the previously used estimators.

Likelihood ratio tests based on the three-parameter model were also shown to have the nominal type 1 error rate, and to be more powerful than most other tests for a given sample size.

When the new model was applied to existing meristic character data from fishes, there was not a substantial difference in the results of the analysis from those described by the original researchers. This was not surprising since most characters were varaible enough that

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the bias should not have been severe, but other factors could also have contributed to making the causes of developmental instability obscure. Among these factors are small sample sizes, samples which contain a mix of cohorts with potentially different levels of developmental instability, counting errors, and lack of measurements of the true factors contributing to the asymmetry.

A large data set for pectoral fin rays of east coast striped bass stocks indicated that year to year variation in asymmetry is substantial, and potentially greater in magnitude than the variance due to natal river. This year to year variation in fluctuating asymmetry could obscure or fonfound differences that are due to natal stream in mixed-age samples.

RECOMMENDATIONS FOR FUTURE

The true value of the three-parameter model for advancing our understanding of developmental instability and its causes can only be ascertained through further applications of the model. To facilitate its use, I have begun packaging the estimation and testing algorithms for use by other researchers and will make them available upon request. Without access to the estimation algorithms, it is unlikely that many other researchers will expend the considerable effort required to develop these programs. By making the programs freely available, I hope that the community of phenogenetics researchers will be able to use

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and further validate the methods.

There are also several additional avenues of inquiry that can be explored using the techniques presented here. First, it appears from the initial examination of previous data, that at least two of the three parameters (σ_L and σ_a) may be useful when examining developmental stability. Further exploration of both parameters may provide insight into stress and its causes. In many of the past data sets σ_L and σ_a appear to vary together. This pattern could result from populations that control the character less tightly being more sensitive to developmental instability, or it could arise if a stressor affects both the variability of the character mean and the coordination of growth between the two sides. Experiments in which a stressor that does not change the character mean is applied in a broad range of concentrations could be very informative in understanding the relationship between σ_L and σ_a. Examples of suitable experiments are the exposure of grunion eggs and larvae to DDT (Valentine et al. 1973) and the exposure of Drosophila to lead and benzene (Graham et al. 1992). I have contacted Graham and will be working with him on analysis of these data in the near future.

Another area that needs further development is the incorporation of counting errors into the estimation algorithms. So far, I have only examined the bias to the estimates that result when known degrees of error are added to data that otherwise perfectly fit the conceptual model.

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Enhancement of the algorithms to incorporate replicate counts for individual organisms when they are available, calculate error probabilities, and remove this variability from the estimates of developmental instability would seem to be a needed area for further development. I have been considering how to do this, but have not yet begun enhancement of the computer code.

Although the underlying theory of the three-parameter model fits morphometric characters as well as it does meristic characters, the algorithms presently will handle only integer data that arises from meristic characters. Conceptually, morphometric data could be converted to integers by scaling up the values, but as they are now constructed, the algorithms would run extremely slowly due to the large number of matrix cells that would be produced by the rescaling. Alternatively, the algorithms could be rewritten to handle continuous variables. The algorithms for continuous data would actually be substantially simpler and faster than those required for the integer-valued data because only a single bivariate probability would need to be calculated for each observation in each iteration of the search, instead of the 25 to 625 now required to estimate the 1x1 cell probabilities. However, estimates of μ, σ_L, and σ_a for continuous data are likely to be essentially those that would be produced by a one-way ANOVA using individuals as blocks and using the two sides as replicate observations within blocks.

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A further possible theoretical enhancement to the model would be to incorporate alternative distributions for the developmental instability. One possible explanation for the high frequency of significant kurtosis in the distribution of A_i is that the normal distribution is not the correct distribution of the developmental deviation for some characters. Early in the present research I included a second three-parameter model in which the deviation in the liability due to stress had a double exponential rather than a normal distribution. This model appeared superior, i. e. produced lower NLL, to the normal model for some data sets, but the algorithm was very unstable when estimating the double exponential q parameter. Further work on the double exponential estimation algorithm could produce a better-fitting model for at least some of the data.

Finally, the literature on fluctuating asymmetry in fishes is replete with field studies in which developmental instability of different populations is compared. The putative cause of differences is in some cases measured, and in others only inferred. At present, only a handful of experiments have been conducted on fish, thus the field studies often lack a sound causal basis for the effects being postulated. While fluctuating asymmetry shows promise as being a quick, easy indicator of stress in fish populations, we don't understand how, or even whether, many of the potential stressors we might want to examine actually affect stability of meristic characters. Additional

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laboratory studies, in which stocks of known genetic background are exposed to varying levels of contaminants (or other stresses) are sorely needed. These could eventually lead to a standardized fluctuating asymmetry bioassay similar to those already developed using fathead minnows. The fluctuating asymmetry bioassay would potentially be more sensitive than the standard tests, since the endpoints being examined occur long before toxicant induced mortality.

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STATISTICAL APPENDIX

Since the double geometric distribution is not found in common statistical reference texts, some of the statistical properties of the distribution and associated estimators and tests are presented for the readers' convenience.

Let X be a discrete random variable with a probability distribution defined as:

$$P(X=x) = (1-q)q^{|x|} \quad \text{for } x = 0$$

$$= \tfrac{1}{2}(1-q)q^{|x|} \quad \text{for } x \neq 0$$

$$0 \leq q \leq 1$$

The probability that $X = 0$ is the same as that of the geometric distribution, but for $X \neq 0$, the probability is one-half of the geometric probability that $X=|x|$.

The mean μ and variance σ^2 are given by:

$$\begin{aligned} \mu &= E(X) \\ &= \sum_{x=-\infty}^{-1} \frac{x}{2}(1-q)\,q^{|x|} + 0 + \sum_{x=1}^{\infty} \frac{x}{2}(1-q)\,q^{|x|} \\ &= \sum_{x=1}^{\infty} \frac{x-x}{2}(1-q)\,q^{|x|} \\ &= 0 \end{aligned}$$

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$$\begin{aligned}
\sigma^2 &= E(X-\mu)^2 = E(X^2) \\
&= \sum_{x=-\infty}^{\infty} \frac{x^2}{2}(1-q)\,q^{|x|} \\
&= \frac{(1-q)}{2}\sum_{x=-\infty}^{\infty} x^2 q^{|x|} \\
&= (1-q)\sum_{x=0}^{\infty} x^2 q^{|x|} \\
&= q + 3q^2 + 5q^3 + 7q^4 + 9q^5 + \cdots
\end{aligned}$$

The maximum likelihood estimate of q is found from:

$$L = \left(\frac{1}{2}\right)^m (1-q)^n q^{\sum |x|}$$

where L = likelihood function

m = number of non-zero observations in the sample

n = total number of observations in the sample

Taking the derivative of the the likelihood function with respect to the parameter q, and setting the derivative equal to 0:

$$\frac{dL}{dq} = \left(\frac{1}{2}\right)^m \left[-n(1-q)^{n-1} q^{\sum |x|} + (1-q)^n \sum |x| q^{\sum |x|-1}\right] = 0$$

The maximum likelihood estimate is found by solving for q.

$$\hat{q} = \frac{\sum_n |x|}{n + \sum_n |x|}$$

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The estimator $\hat{q}$ is shown to be unbiased since its expected value is equal to the parameter q:

$$E(\hat{q}) = \frac{\sum_{n} E|x|}{n + \sum_{n} E|x|} = \frac{nE|x|}{n + nE|x|} = \frac{E|x|}{1 + E|x|}$$

$$= \frac{\frac{1}{2}\sum_{x=-\infty}^{\infty} |x|\,(1-q)\,q^{|x|}}{1 + \frac{1}{2}\sum_{x=-\infty}^{\infty} |x|\,(1-q)\,q^{|x|}} = \frac{(1-q)\sum_{x=0}^{\infty} xq^{x}}{1 + (1-q)\sum_{x=0}^{\infty} xq^{x}}$$

$$= \frac{q+q^{2}+q^{3}+q^{4}+q^{5}\cdots}{1+q+q^{2}+q^{3}+q^{4}\cdots} = \frac{q(1+q+q^{2}+q^{3}+q^{4}\cdots)}{1+q+q^{2}+q^{3}+q^{4}\cdots}$$

$$= q$$

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COLLATED BIBLIOGRAPHY

Ames, L. J., J. D. Felley, and M. H. Smith. 1979. Amounts of asymmetry in centrarchid fish inhabiting heated and nonheated reservoirs. Transactions of the American Fisheries Society 108:489-495.

Angus, R. A. 1982. Quantifying fluctuating asymmetry: Not all methods are equivalent. Growth 46:337-342.

Astauroff, B. L. 1930. Analyse der erblichen Störungsfälle der bilateralen Symmetrie im Zusammenghang mit der selbständigen Variabilität ähnlicher Strukturen. Zeitschr. Indukt. Abst. Vererb. 55:183-262.

Bell, M. A. 1982. Differentiation of adjacent stream populations of threespine sticklebacks. Evolution 36:189-199.

Blaxter, J. H. 5. 1984. Ontogeny, systematics and fisheries. American Society of Ichthyologists and Herpetologists Special Publication 1:1-6.

Boreman, J., and R. J. Klauda. 1988. Distributions of early life stages of striped bass in the Hudson River estuary, 1974-1979. American Fisheries Society Monograph 4:53-58.

Buckley, L. J., T. A. Halavik, G. C. Laurence, S. J. Hamilton, and P. Yevich. 1985. Comparative swimming stamina, biochemical composition, backbone mechanical properties, and histopathology of juvenile striped bass from rivers and hatcheries of the eastern United States. Transactions of the American Fisheries Society

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114:114-124.

Carline, R. F. and M. V. Lawal. 1985. Contaminants and bilateral asymmetry in yellow perch. Environmental Toxicology and Chemistry 4:543-547.

Chapman, R. W. 1990. Mitochondrial DNA analysis of striped bass populations in Chesapeake Bay Copeia 1990:355-366.

Clarke, G. M. 1992. Fluctuating asymmetry: A technique for measuring developmental stress of genetic and environmental origin. Acta Zoologica fennica 191:31-35.

Clarke, G. M. 1994. The genetic basis of developmental stability. I. Relationships between stability, heterozygosity and genomic coadaptation. Pages 17-25 In T. A. Markow (ed.) Developmental Instability: Its Origins and Evolutionary Implications. Kluwer Academic Publishers. Netherlands.

Clarke, G. M. 1995. Relationships between developmental stability and fitness: Applicaton for conservation biology. Conservation Biology 9(1):18-24.

Dey, W. P. 1981. Mortality and growth of young-of-the-year striped bass in the Hudson River estuary. Transactions of the American Fisheries Society 110:151-157.

Eldridge, M. B., J. Whipple, and D. Eng. 1979. Endogenous energy sources as factors affecting mortality a nd development in striped bass (Morone saxatilis) eggs and larvae. In Early Life History of fish, Volume II. K.

Reproduced with permission of the copyright owner. Further reproduction prohibited without permission.

Sherman and R. Lasker (eds.) Springer-Verlag.

Emlen, J. M., D. C. Freeman and J. H. Graham. 1993. Nonlinear growth dynamics and the origin of fluctuating asymmetry. Genetica 89:77-96.

Escós, J., C. L. Alados, J. M. Emlen, and S. Alderstein. 1995. Developmental instability in the Pacific hake parasitized by Myxosporeans Kudoa spp. Transactions of the American Fisheries Society 124:943-945.

Felley, J. 1980. Analysis of morphology and asymmetry in bluegill sunfish (Lepomis macrochirus) in the southeastern United States. Copeia 1980:18-29.

Finger, S. E., and J. S. Bulak. 1988. Toxicity of water from three South Carolina rivers to larval striped bass. Transactions of the American Fisheries Society 117:521-528.

Freeman, D. C., J. H. Graham, and J. M. Emlen. 1993. Developmental stability in plants: symmetries, stress and epigenesis. Genetica 89:97-119.

Freeman, D. C., J. M. Emlen, J. H. Graham, R. L. Mara, M. Tracy, and C. L. Alados. 1995. Developmental instability as a bioindicator of ecosystem health. Pages 170-177. In Proceedings: shrubland ecosystem dynamics in a changing environment.

Goodyear, C. P. 1985. Toxic materials, fishing, and environmental variation: simulated effects on striped bass population trends. Transactions of the American Fisheries Society 114:107-113.

Reproduced with permission of the copyright owner. Further reproduction prohibited without permission.

Graham, J. H. and J. D. Felley. 1985. Genomic coadaptation adn develoopmental stability within introgressed popuatoins of *Enneacanthus gloriosus* and *E. obesus* (Pisces, Centrarchidae). Evolution 39:104-114.

Graham, J. H. 1992. Genomic coadaptation and developmental stability in hybrid zones. Acta Zoologica Fennica 191:121-131.

Graham, J. H., D. C. Freeman, and J. M. Emlen. 1993. Developmental Stability: A sensitive indicator of populations under stress. Pages 136-158 In Landis, W. G., J. S. Hughes, and M. A. Lewis (eds.) Environmental Toxicology and Risk Assessment, ASTM STP 1179. Philadelphia.

Graham, J. H., J. M. Emlen, and D. C. Freeman. 1993a. Developmental stability and its applications in ecotoxicology. Ecotoxicology 2:175-184.

Graham, J. H., D. C. Freeman and J. M. Emlen. 1993b. Antisymmetry, directional asymmetry, and dynamic morphogenesis. Genetica 89:121-137.

Graham, J. H., K. E. Roe, and T. B. West. 1993. Effects of lead and benzene on the developmental stability of *Drosophila melanogaster*. Ecotoxicology 2:185-195.

Graham, J. H., J. M. Emlen, D. C. Freeman, L. J. Leamy, and J. A. Kieser. 1998. Directional asymmetry and the measurement of developmental instability. Journal of the Linnean Society 64:1-16.

Grove, T. L., T. J. Berggren, and D. A. Powers. 1976. The

Reproduced with permission of the copyright owner. Further reproduction prohibited without permission.

use of innate tags to segregate spawning stocks of striped bass, Morone saxatilis. Pages 166-176 in M. Wiley, editor. Estuarine Processes. Academic Press, New York.

Hall, L. W. 1987. Acidification effects on larval striped bass, Morone saxatilis, in Chesapeake Bay tributaries-a review. Water, Air and Soil Pollution 35:87-96.

Hall, L. W., Jr. 1991. A synthesis of water quality and contaminants data on early life stages of striped bass, Morone saxatilis. Reviews in Aguatic Sciences 4:261-288.

Heitzman, R. A. 1982. DDT, Aroclor 1254, and mercuric chloride as potential inducers of bilateral asymmetry in the fathead minnow (Pimephales promelas). M. S. Thesis. The Ohio State University, Columbus.

Hilborn, R. and M. Mangel. 1997. The Ecological Detective: confronting models with data. Princeton University Press. Princeton.

Hillier, F. S. and G. J. Lieberman. 1980. Introduction to Operations Research. Third Edition. Holden-Day, Inc. Oakland.

Hubbs, C. L. and L. C. Hubbs. 1945. Bilateral asymmetry and bilateral varaiation in fishes. Papers form the Michigan Academ of Science, Arts and Letters 30:229-311.

Hubbs, C. L., and K. F. Lagler. 1947. Fishes of the Great Lakes Region. Bulletin of the Cranbrook Institute of

Reproduced with permission of the copyright owner. Further reproduction prohibited without permission.

Science 26.

Hubert, W. A. and C. B. Alexander. 1995. Observer variation in counts of meristic traits affects fluctuating asymmetry. North American Journal of Fisheries Management 15:156-158.

Jagoe, C. H. and T. A. Haines. 1985. Fluctuating asymmetry in fishes inhabiting acidified and unacidified lakes. Canadian Journal of Zoology 63:130-138.

Kendall, A. W., Jr., E. H. Ahlstrom, and H. G. Moser. 1984. Early life history stages of fish and their characters. American society of Ichthyologists and Herpetologists Special Publication 1:11-22.

Leary, R. F. and F. W. Allendorf. 1989. Fluctuating asymmetry as an indicator of stress: implications for conservaton biology. Trends in Ecology and Evolution 4:214-217.

Leary, R. F., F. W. Allendorf, and K. L. Knudsen. 1992. Genetic, environmental, and developmental causes of meristic variation in rainbow trout. Acta Zoologica Fennica 191:79-95.

Leung, B. and M. R. Forbes. 1997. Modelling fluctuating asymmetry in relation to stress and fitness. Oikos 78:397-405.

Mather, K. 1953. Genetical control of stability in develpoment. Heredity 7:297-336.

Markow, T. A. 1994. Developmental Instability: Its Origins and Evolutionary Implications. Kluwer Academic

Reproduced with permission of the copyright owner. Further reproduction prohibited without permission.

Publishers. Netherlands.

Markowski, C. A. and E. P. Markowski. 1990. Conditions for the effectiveness of a preliminary test of variance. The American Statistician 44:322-326.

Matthews, W. J. 1990. Spatial and temporal variation in fishes of riffle habitats: a comparison of analytical approaches for the Roanoke River. American Midland Naturalist 124:31-45.

Mehrle, P. M., T. A. Haines, S. Hamilton, J. L. Ludke, F. L. Mayer, and M. A. Ribick. 1982. Relationship between body contaminants and bone development in east-coast striped bass. Transactions of the American Fisheries Society 111:231-241.

Møller, A. P. 1997. Developmental stability and fitness: a review. The American Naturalist 149:916-932.

Moran, P, J. I. Izquierdo, A. M. Pendas, and E. Garcia-Vazquez. 1997. Fluctuating asymmetry and isozyme variation in Atlantic salmon: Relation to age of wild and hatchery fish. Transactions of the American Fisheries Society 126:194-199.

Morgan, R. P., II, V. J. Rasin, Jr., and R. L. Copp. 1981. Temperature and salinity effects on development of striped bass eggs and larvae. Transactions of the American Fisheries Society 110:95-99.

Mood, A. M., F. A. Graybill, and D. C. Boes. 1974. Introduction to the theory of statistics. Third Edition. McGraw-Hill, New York.

Reproduced with permission of the copyright owner. Further reproduction prohibited without permission.

Nilsson, J. 1994. Energetic stress and the degree of fluctuating asymmetry: implications for a long-lasting, honest signal. Evolutionary Ecology 8:248-255.

Otto, R. 5. 1975. Isoenzyme sytems of the striped bass (Morone saxatilis) and congeneric percichthyid fishes. Doctoral dissertation. University of Maine, Orono.

Palmer, A. R. 1986. Inferring relative levels of genetic variability in fossils: the link between heterozygosity and fluctuating asymmtery. Paleobiology 12(1):1-5.

Palmer, A. R. and C. Strobeck. 1986. Fluctuating asymmetry: measurement, analysis, patterns. Annual Review of Ecology and Systematics 17:391-421.

Palmer, A. R. 1994. Fluctuating asymmetry analyses: A primer, pp.335-364. In T. A. Markow (ed.) Developmental Instability: Its Origins and Evolutionary Implications, Kluwer, Dordrecht, Netherlands.

Palmer, A. R. and C. Strobeck. 1992. Fluctuating asymmetry as a measure of developmental stability: Implications of non-normal distributions and power of statistical tests. Acta Zoologica Fennica 191:55-70.

Parsons, P. A. 1962. Maternal age and developmental variability. Journal of Experimental Biology 39:251-260.

Parsons, P. A. 1990. Fluctuating asymmetry: an epigentic measure of stress. Biological Review 65:131-145.

Reproduced with permission of the copyright owner. Further reproduction prohibited without permission.

Pomory, C. M. 1997. Fluctuating asymmetry: biological relevance or statistical noise. Animal Behavior 53:225-227.

Quattro, J. M. and R. C. Vrijenhoek. 1989. Fitness differences among remnant populations of the endangered Sonoran topminnow. Science 245:976-978.

Reeve, E. C. R. 1960. Some genetic tests on asymmetry of sternopleural chaeta number in Drosophila. Genetic Research 1:151-172.

Rohde, P. A., T. Amundsen and P. Fiske. 1997. Fluctuating asymmetry, mate choice and experimental designs. Animal Behavior 54:1030-1033.

Rutherford, E. S., and E. D. Houde. 1995. The influence of temperature on cohort-specific growth, survival, and recruitment of striped bass, Morone saxatilis, larvae in Chesapeake Bay. U.S. National Marine Fisheries Service Fishery Bulletin 93:315-332.

SAS. 1987. SAS/STAT guide for personal computer. SAS Institute, Cary, North Carolina.

Scheffe', H. 1959. The analysis of variance. John Wiley & Sons, New York.

Sidell, B. D., R. G. Otto, D. A. Powers, M. Karweit, and J. Smith. 1980. Apparent genetic homogeneity of spawning striped bass in the Upper Chesapeake Bay. Transactions of the American Fisheries Society 109:99- 107.

Simmons, L. W., J. L. Tomkins and J. T. Manning. 1995. Sampling bias and fluctuating asymmetry. Animal

Reproduced with permission of the copyright owner. Further reproduction prohibited without permission.

Behavior 49:1697-1699.

Sokol, R. R. and F. J. Rohlf. 1993. Biometry. Third Edition. W. H. Freeman, San Francisco.

Sokal, R., and J. Rohlf. 1995. Biometry: the Principles and Practice of Statistics in Biological Research. Third Edition W. H. Freeman Company, New York.

Soulé, M. E. 1967. Phenetics of natural populations. II. Asymmetry and evolution in a lizard. American Naturalist 101:141-160.

Soulé, M. E. 1979. Heterozygosity and developmental stability: Another look. Evolution 33:396-401.

Summers, J. K., and K. A. Rose. 1987. The role of interactions among environmental conditions in controlling historical fisheries variability. Estuaries 10:255-266.

Sumner, J. L. and R. R. Huestis. 1921. Bilateral asymmetry and its relation to certain problems of genetics. Genetics 6:445-485.

Swaddle, J. P. 1997. Experimental design and signalling properties of fluctuating asymmetry. 54:1034-1037.

Swaddle, J. P., M. S. Witter and I. C. Cuthill. 1994. The analysis of fluctuating asymmetry. Animal Behavior 48:986-989.

Swain, M. W. 1987. A problem with the use of meristic characters to estimate developmental stability. The American Naturalist 129:761-768.

Tebb, G. and J. M. Thoday. 1954. Stability in development

Reproduced with permission of the copyright owner. Further reproduction prohibited without permission.

and relational balance of X-chromosomes in Drosophila melanogaster. Nature 174:1109-1110.

Thoday, J. M. 1955. Balance, heterozygosity and developmental stability. Cold Spring Harbor Symposium Quant. Biology 22:318-326.

Thomas, R. and R. Poulin. 1997. Using randomization techniques to analyse fluctuating asymmetry. Animal Behavior 54:1027-1029.

Toft, C. A. 1990. Reply to Seaman and Jaeger: an appeal to common sense. Herpetologica 46:357-361.

Tracy, M., D. C. Freeman, J. M. Emlen, J. H. Graham, and R. A. Hough. 1995. Developmental instability as a biomonitor of environmental stress. Pages 313-337 In Biomonitors and Biomarkers as Indicators of Environmental Change. Butterworth, F. M. et al. (eds.) Plenum Press, New York.

Ulanowicz, R. E., and T. T. Polgar. 1980. Influences of anadromous spawning behavior and optimal environmental conditions upon striped bass (Morone saxatilis) year-class success. Canadian Journal of Fisheries and Aquatic Sciences 37:143-153.

Uphoff, J. H., Jr. 1989. Environmental effects on survival of eggs, larvae, and juveniles of striped bass in the Choptank River, Maryland. Transactions of the American Fisheries Society 118:251-263.

Valentine, D. W. and M. Soulé. 1973. Effect of p,p'-DDT on developmental stability of pectoral fin rays in the

Reproduced with permission of the copyright owner. Further reproduction prohibited without permission.

grunion, Leuresthes tenuis. Fishery Bulletin 71:921-926.

Valentine, D. W. and M. E. Soulé, and P. Samallow. 1973. Asymmetry analysis in fishes: A possible statistical indicator of environmental stress. Fishery Bulletin 71:357-370.

Van Valen, L. 1962. A study of fluctuating asymmetry. Evolution 16:125-142.

Vladykov, V. D. 1934. Environment and taxonomic characters of fishes. Transactions of the Royal Canadian Institute 20:99-140.

Vrijenhoek, R. C. and S. Lerman. 1982. Heterozygosity and developmental stability under sexual and asexual breeding systems. Evolution 36:768-776.

Waldman, J. R., R. A. Richards, W. B. Schill. I. Wirgin, and M. C. Fabrizio. (1997) An empirical comparison of stock identification techniques applied to striped bass Morone saxatilis. Transactions of the American Fisheries Society. 126:369-385.

Watson, P. J. and R. Thornhill. 1994. Fluctuating asymmetry and sexual selection. Trends in Ecology and Evolution 9:21-25.

Westin, D. T., C. E. Olney, and B. A. Rogers. 1985. Effects of parental and dietary organochlorines on survival and body burdens of striped bass larvae. Transactions of the American Fisheries Society 114:125-136.

Reproduced with permission of the copyright owner. Further reproduction prohibited without permission.

Wiener, J. G. and P. J. Rago. 1987. A test of fluctuating asymmetry in bluegills (Lepomis macrochirus Rafinesque) as a measure of pH-related stress. Environmental Pollution 44:27-36.

Wirgin, I. I., P. Silverstein, and J. Grossfield. 1990. Restriction endonuclease analysis of striped bass mitochondrial DNA: the Atlantic coastal migratory stock. American Fisheries Society Symposium 7:475-491.

Zakharov, V. M. and V. V. Zyuganov. 1980. Assessment of asymmetry of bilateral characters as a population characteristic. Ékologiya 1:10-16.

Zakharov, V. M. and J. H. Graham. 1992a. Developmental stability in natural populatons. Introduction. Acta Zoologica Fennica 191:4-5.

Zakharov, V. M. and J. H. Graham. (eds.) 1992b. Developmental Stability in Natural Populations. Acta Zoologica Fennica No. 191.

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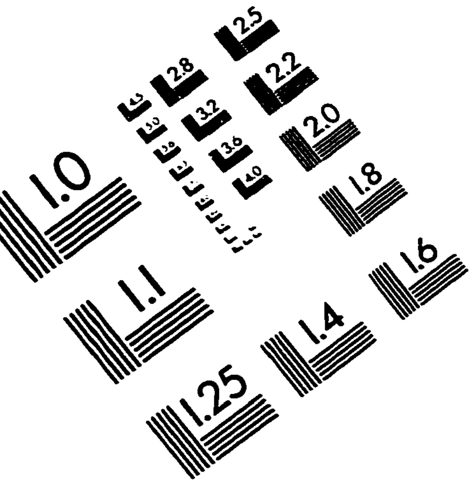
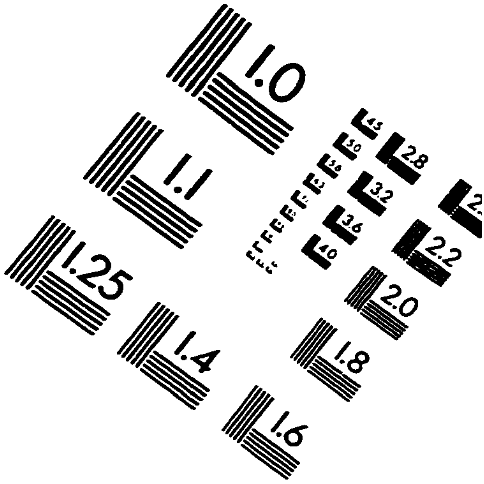
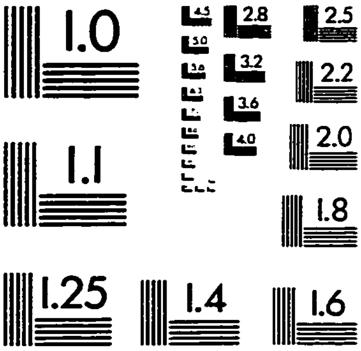
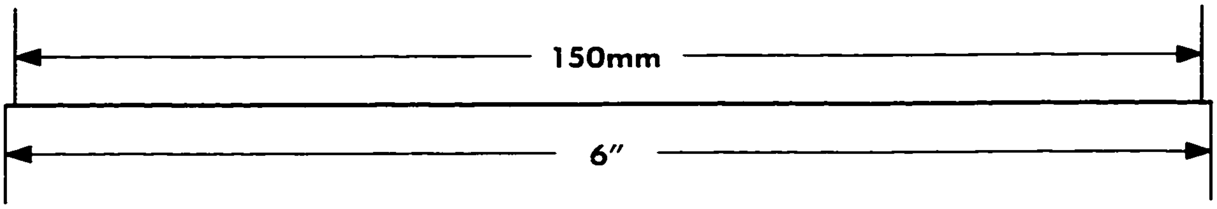
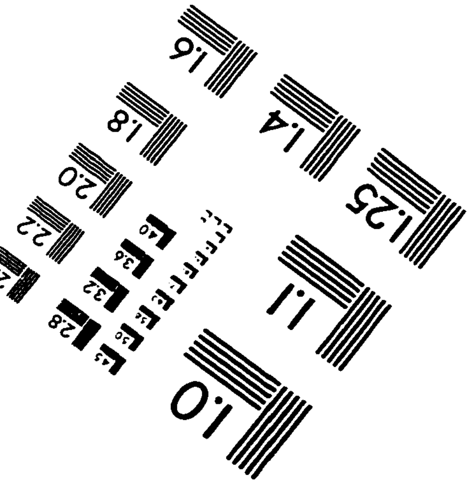
IMAGE EVALUATION TEST TARGET (QA-3)

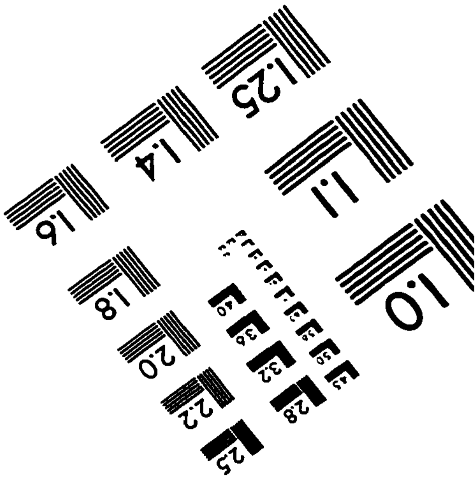












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