

Intraspecific Variation among Emerald Shiners (*Notropis atherinoides*)  
of the Missouri River

BY

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Intraspecific Variation among Emerald Shiners (*Notropis atherinoides*)  
of the Missouri River

This dissertation is approved as a creditable and independent investigation by a candidate for the Doctor of Philosophy degree and is acceptable for meeting the dissertation requirements for this degree. Acceptance of this dissertation does not imply that the conclusions reached by the candidate are necessarily the conclusions of the major department.

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## Abstract

Intraspecific Variation among Emerald Shiners (*Notropis atherinoides*)  
of the Missouri River

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Fishes of the Missouri River are separated by six dams and impoundments and inhabit a variety of natural and engineered habitat types. Concern over the effects of river alteration led to an investigation of whether the Missouri River fish have been segregated into differentiated sub-populations. Emerald shiners (*Notropis atherinoides*) were chosen as a representative species because of their wide distribution, record of morphologic and meristic plasticity, and life history characteristics. These conditions led to the hypothesis that the differing and disjunct habitats of the Missouri River may be acting as selective pressures to produce distinct sub-populations within the Missouri River. To test this hypothesis I designed a set of four analyses to determine if 1) there were any intraspecific differences among sites, 2) the differences were related to a genetic population structure, 3) the differences were correlated with habitat types, and 4) if the differences provided a measurable performance advantage.

Emerald shiners were collected from both reservoir and river habitats.

Differences in body form were measured using a box-truss protocol. This created a 2-dimensional projection of the fish using fixed body landmarks to define body shape. A principal components analysis of these data indicated that posterior body length, eye position, and head length account for the variation seen among sites. Other morphological characters such as jaw width ( $P < 0.001$ ), head depth ( $P < 0.001$ ), and eye diameter ( $P < 0.001$ ), provided more discrimination between sites. Larger eye diameters were associated with lentic and low turbidity sites. Shape was highly variable among mainstem Missouri River sites, but less variable among lentic and tributary sites. Local adaptation may occur on a small scale, but large scale morphometry appears to be highly variable thus precluding the need for specialized forms.

Meristic values (counts of vertebrae, fin rays, scales, teeth, taste buds, etc.) are governed by genotype, but can vary during embryonic development in response to temperature, sunlight, and stress. Naturally, a predictable longitudinal gradient or random insignificant variation should be seen. When conditions change abruptly or are altered, marked interruptions in the gradient and significant variation in meristic counts may arise. I measured dorsal, anal, pectoral, and pelvic fin rays and vertebrae on emerald shiners from all portions of the Missouri River. Only the pectoral fin ray numbers (9-14) and vertebrae counts (35-43) significantly differed among sites ( $P < 0.001$ ). Pectoral ray numbers seemed to be disrupted by the presence of reservoirs, but vertebrae number followed a gradual increase with increasing latitude. These results suggest that local conditions can have site-specific effects on some characteristics during development, but may leave others unaffected.

To measure genetic variation I collected 30 individuals from four mainstem Missouri River sites, a site in the Yellowstone River, and an outgroup from Lake Erie. Using 28 surveyed loci, I found 14 of them to be polymorphic. The Yellowstone River was 2-4 magnitudes different from its Missouri River neighbors, identifying the Yellowstone River as genetically isolated to a degree from the rest of the Missouri River mainstem sites. The Montana site was more genetically similar to the Missouri site than to its neighboring Yellowstone River site. This relationship suggests a degree of reproductive isolation between the Yellowstone River and the entire mainstem Missouri River. The mainstem Missouri River was genetically panmictic (genetically mixed through immigration and emigration) from headwaters to mouth and has not been measurably affected or segregated genetically by impoundments at this point in time. These genetic data do not support the pattern of differing characteristics associated with specific habitats seen in either the morphometric or meristic analyses. The logical conclusion then is that the physical variation in emerald shiners was a result of intraspecific plasticity unrelated to genetic isolation or drift.

I next used a laminar flow tunnel to determine the functional significance of physical and behavioral changes among emerald shiners from different habitats. Fish were swum for five minutes at increasing velocity intervals until they were fatigued. Fish of the same size class were used from each site. Performance varied erratically among individuals from each site ( $45 < U_{crit} < 93$  cm/sec), but did not differ significantly between sites, indicating that neither the habitat in which they were reared nor their differences in physical characteristics provided any measurable difference in swimming performance.

Intraspecific plasticity may be a response to differences in habitat, but the differences do not seem to substantially affect the functionality or performance potential of fish from either site. The alterations to the Missouri River appear to have affected certain physical aspects of emerald shiners, however the inherent plasticity of the species seems to allow it to adapt or react locally without the loss of genetic integrity. These integrated analyses then support the conclusion that selective pressures in different habitats may cause change in morphometric and meristic characters, but are likely attributable to local adaptive plasticity rather than genetic drift resulting from sub-population formation.

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## Chapter 1

### Intraspecific Variation: An Integrated Approach to Understanding the Basis, Cause, and Results of Change in a Species

#### ABSTRACT

The Missouri River has been altered from its natural state by dam construction and channelization. The native fish fauna which once inhabited the natural river have had to adapt to the new conditions in many different ways. Physical characteristics and behaviors were each subject to transformations when conditions necessitated the change. Differences in habitat may have directly caused differentiation in phenotypes and indirectly caused differentiation in genotypes in fishes. This investigation was designed to determine the existence and degree of changes in several characteristics of a species in response to habitat alterations in the Missouri River. The emerald shiner (*Notropis atherinoides*) was selected to examine how a single species that once occupied the entire Missouri River has been affected by extreme habitat changes. The emerald shiner is noted for being morphologically plastic and was expected to display more pronounced changes than most species. Meristic counts were done to determine patterns in structural

development throughout the river. Morphological measurements to determine shape distortions were done using a truss analysis and associated principal components tests. Allozyme analyses were conducted to determine if genetic separation between groups of shiners within the Missouri River has occurred. Finally, stamina testing was done in a controlled flow tunnel to determine if differences seen in the fish actually equated to ecological or physiological advantages. These four analyses were performed to reveal either a uniform, grouped, random, or graded response. The responses and their inter-relationships signified the type of association present among the emerald shiners from different regions of the river and which deterministic forces served to differentiate them.

## INTRODUCTION

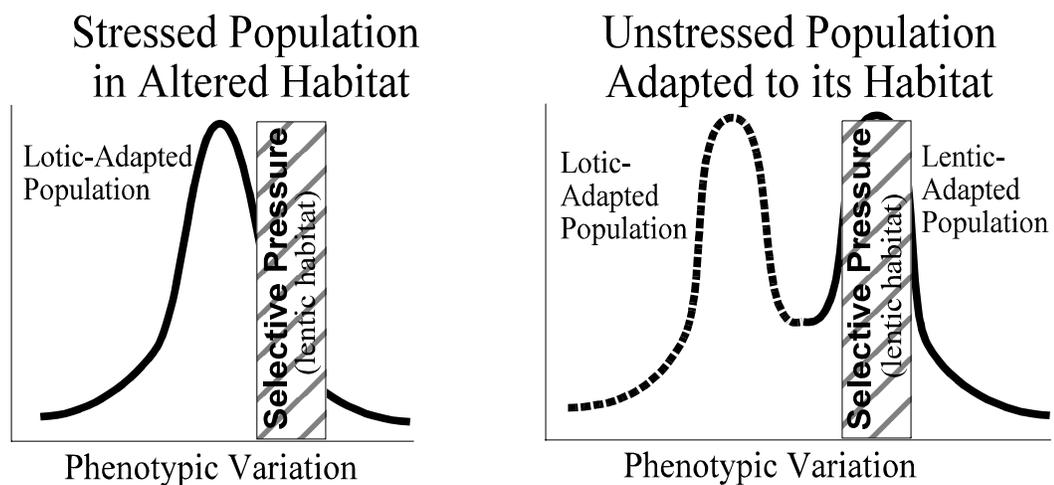
Organisms have increasingly become an important tool used in evaluating the effects of differences in and changes to habitat (Karr et al. 1986; Munkittrick and Dixon 1989; Balon 1995). Rather than just measuring physical habitat differences, communities, or populations, individual organisms are being used to understand the ecological meaning and influence that habitat differences can have on species. The characteristics of an organism, population, or community can help us understand the impact of environmental changes by recognizing how species have adapted to cope with these changes. Habitats are constantly and naturally changing in gradual and usually benign ways. Sometimes however, a habitat may be changed dramatically through a catastrophic event such as earthquakes, volcanoes, glaciation, or uncommonly large floods. These changes force populations to either adapt immediately or be extirpated

from that portion of their range (Mayden 1988b). In addition to natural events, large scale anthropogenic changes of the landscape can have similar effects on species. The effects on species caused by the construction of dams and subsequent regulated flows has been shown in many studies (Fisher and LaVoy 1972; Cushman 1985; Stanford et al. 1988; Troelstrup and Hergenrader 1990; Weisburg and Burton 1993; Ligon et al. 1995; Wolf et al. 1996; Copp 1997). The Missouri River and its reservoir and flow control system is an example of large scale habitat alteration.

The present state of the river is far from what it used to be and from what it would be naturally if not controlled. The Missouri River is 3768 km long from its source in Three Forks, Montana to its confluence with the Mississippi River in Saint Louis, Missouri. Historically, the Missouri River began as a swift gravel and cobble bed river within a channel highly confined by bluffs and ended as a wide, floodplain river. Although the Missouri River remains the same at its headwaters, a downstream progression now finds a series of six large dams and reservoirs. The lowest third of the Missouri River is now highly confined by dikes and levees and nearly devoid of meanders and sandbars. These physical changes to the river were completed in only a few decades.

The biota inhabiting the river cannot change their characteristics in response to habitat alteration within that same time frame. A stressor was imposed on populations forcing them to cope with conditions under which they did not evolve. Natural variation in a population allows for a portion to survive and reproduce, yet selects against a small portion as well, preventing their reproduction and further contribution of genes to the population. The remaining individuals become part of a transitional population that

moves toward a set of characteristics that will better enable their existence under the new conditions. Eventually, the population will reach an equilibrium where the characteristics of the population better match the conditions of the habitat. At that point, the forces of natural selection stop favoring one tail of the natural variability in the population as the mean of natural variation shifts to match the selective pressure (Figure 1). These processes occur constantly in nature but usually at a very slow and gradual pace. Changes in characteristics are typically seen to change over hundreds and thousands of generations



**Figure 1.** A conceptual diagram showing how a selective pressure, through natural selection, can produce a distinctly different phenotype, better adapted to local environmental conditions. The bi-modal curve on the right represents the shift to a new phenotype which may either replace or coexist with the original phenotype depending on whether original environmental conditions are lost or maintained.

because selective pressures usually change slowly and gradually, not drastically. However, when a dramatic habitat change occurs during the life of an individual or during the course of only a few generations, the population is forced to adapt more quickly.

Adaptation is easier for some species than for others. The ability to adapt depends on the physical and behavioral characteristics of a species. Physically, species that have a general or non-descript body form can adapt to a wider range of conditions than can a species with a very specialized body form (Balon et al. 1986). Behaviorally, species thought of as generalists are more able to cope with changes than species classified as specialists. These differences can be considered as degrees of tolerance. The greater tolerance a species has for diverse conditions, the more able it will be to adapt when conditions change. Strauss (1987) even proposed the idea of a “morphological space” in which a species is able to display variable phenotypes in response to environmental stimuli.

Emerald shiners were chosen as a species to focus on for several important reasons. Emerald shiners have a record of showing great morphometric and meristic plasticity (Bailey and Allum 1962; Flittner 1964). The numbers of countable structures on their bodies and their overall shape vary substantially throughout their range (Scott and Crossman 1973). Emerald shiners are also abundant throughout the warm-water portion of the Missouri River. They are common from above Ft. Peck Reservoir to the confluence with the Mississippi River (Young et al. 1997). This distribution covers all the habitat types of the river. Emerald shiners were also recorded in the river prior to

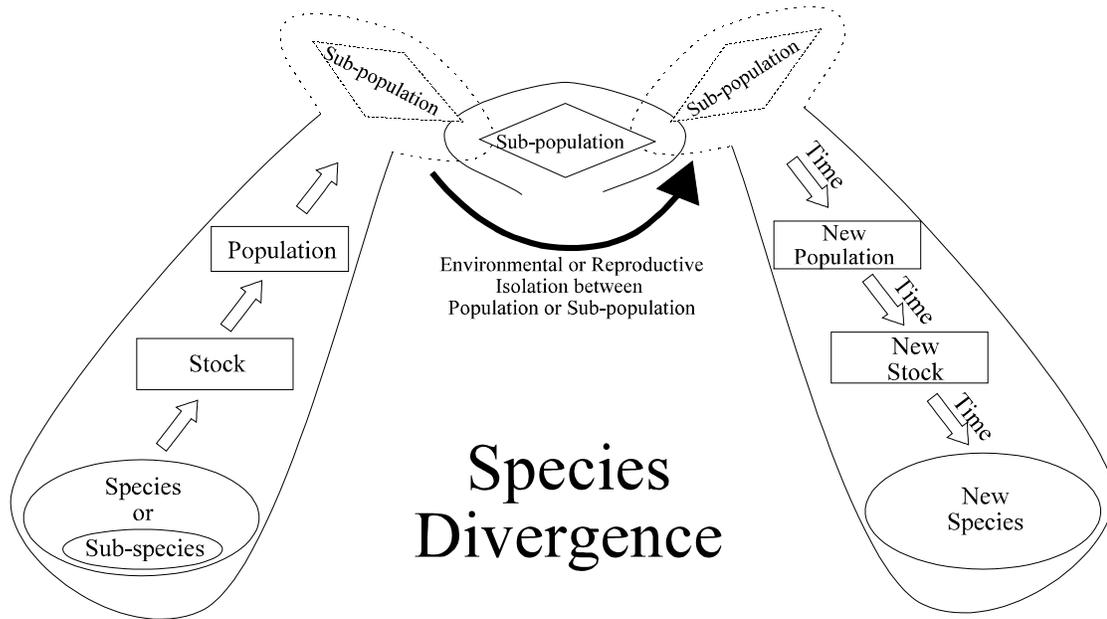
impoundments and channel alterations (Churchill and Over 1938; Bailey and Allum 1962; Pflieger and Grace 1987;). This supports the assumption that the current populations are descendants of native ancestors, not populations introduced from other sources. Finally, emerald shiners have a fast generation time. They are annual spawners, starting the second year of life, and live only three to four years (Fuchs 1967). This spawning cycle insures that between 20 to 25 generations have been produced since impoundment. This many generations improves the likelihood of detecting genetic changes. The short lifespan also insures that the genes of a given individual are only passed on for a short time. Long-lived fish such as shovelnose sturgeon (*Scaphirynchus platorhynchus*) or paddlefish (*Polyodon spathula*) could potentially distribute their genes within a population for decades thereby negating or resetting changes that occur and are incorporated annually. Another reason emerald shiners were a good choice for these types of analyses was because there is no dimorphism between the two sexes except when mature females are gravid with eggs (Flittner 1964). Differences in males and females would require sex identification of each individual in addition to all other characteristics. The uniformity of non-sex related characteristics allows all the fish of a study group to be statistically pooled and treated equally in analyses.

Body shape in fish is related to the environment in which they evolved (Reno 1969). Fast swimming fish and fish that live in flowing waters tend to have more fusiform shaped bodies (Barlow 1961). Fish in static waters often have more compressed and deeper bodies (Hubbs 1941). Sibbing et al. (1994) described how to use these and other attributes to understand the ecomorphology of fishes. The influence of habitat on

body shape is obvious and may be a limiting factor in the persistence of a population. Because a habitat cannot conform to a fish, a fish must conform to its habitat to insure its survival (Wood and Bain 1995). Barlow (1961) stated that morphology is environmentally induced unless otherwise proven and that simple isolation and random divergence is not as important as changes in environmental conditions in causing intraspecific phenotypic divergence. Additionally, Hubbs (1941) stated “Any theory of species formation that fails to explain the intimate tie-up that exists between habitat and characters is at the least incomplete.”

In the case of emerald shiners, I expected that stocks that had been isolated and subjected to new and highly altered habitats would show signs of habitat affecting the form of the fish. Emerald shiners have already been classified by some researchers as comprising two sub-species: one a “lake emerald shiner” and the other a “river emerald shiner” (Hubbs and Lagler 1964). This occurrence of lotic and lentic sub-species has been previously reported in *Gobio gobio* of the Danube River (Bănărescu 1994). However, later researchers argued that the differences seen in emerald shiners did not constitute a subspecies, but that emerald shiners were simply displaying opposite extremes of the natural variation seen within the species (Bailey and Allum 1962). If high variability does exist naturally within the species, then extreme changes in habitat and isolation of different stocks might create the diversity of selection pressures necessary to expose how habitat can mold the form of species (Balon 1992) (Figure 2).

The delineation of criteria that signified my groupings of emerald shiners were subjective yet commonly accepted. In the following studies I defined species groupings



**Figure 2.** A conceptual diagram showing how a sub-population can become isolated from its parent population and form a new population, stock, and eventually species.

in the following ways: 1) *species* are groups of actual or potentially interbreeding natural populations that are reproductively isolated from other groups (Mayr 1963), 2) *sub-species or races* are differentiable from species only in that they can occur with conspecifics yet maintain their own specific genome and phenotype, 3) *strains* most likely arose from stocks, but have differentiated enough to display reproducible physiological, morphological, or performance characteristics that are significantly different from other conspecifics, 4) *stocks or sub-populations* may arise through

partitioning in response to both abiotic and biotic factors or simple isolation, 5) *populations* are defined as groups of organisms occupying a defined area that interbreed regularly and often to maintain a randomly variable gene pool, and 6) *group* simply refers to any definable collection of a species whose actual grouping structure is unknown. Of these definitions, I believe that it is reasonable to assume that the emerald shiners of the Missouri River were once part of either a continuous single population or formed many groups of sub-populations or stocks within the river from Montana to the confluence. This study was designed to find evidence of differences that would resolve groups at a level as great as strains within the river or as little as that seen in a single uniform population. The importance was in the relation of any type of grouping to known habitat characteristics. The presence of definable groups associated with definable habitats and boundaries was thought to be evidence of adaptation.

Despite being impounded and channelized, the Missouri River still flows and carries the same amount of water as it did historically. Water passing through all the impoundments carries a large fish biomass (Walburg 1971). Fishes are entrained through the hydroelectric generators, through the spillway gates, and washed to the river below. This downstream movement, whether intentional or not, insures some mixing of upstream fishes with downstream fishes. The amount of mixing between emerald shiners from different locations was unknown and its effect on homogenization through interbreeding was also unknown. A mixing of two isolated groups could have resulted in several scenarios. The mixing could have been great enough to effectively homogenize the two groups. The mixing might not have been sufficient to cause any changes in the

downstream group because of behavioral separation, disorientation to surroundings, delayed mortality in response to selective pressures, or a simple lack of numbers needed to affect the group. Isolation need not be absolute to allow genetic divergence (Mironovskii 1991). Even with some drift, the bottleneck that allows gene flow may have been sufficiently narrow to effectively isolate two groups. The composition of adjacent groups both genetically and phenotypically was used to determine whether mixing through entrainment provided enough gene flow to homogenize the characteristics of emerald shiners throughout the entire river.

Fish collection was completed without regard for the type of habitat emerald shiners were using at the time of collection. Conspecifics might have inhabited different habitat types during the summer season, but they all are concentrated during the winter in the same habitats. They disperse and select preferred habitats in the spring. I saw no reason to believe that there were distinctively separate outside bend stocks, inside bend stocks, sand bar stocks, or any other habitat type stock of a species within a segment of the river. The only difference might have come from condition factors at the end of the season which differed according to the quality of the habitat occupied during the summer. These differences could have affected measurements such as girth and body depth in the abdominal area, but anatomical placement of structures would not be affected. Morphometric, meristic, and genetic characteristics were all potentially affected by the selective pressures imposed on them throughout the year by habitat, random genetic drift, and mutations.

Other studies have examined species divergence of fishes in several ways.

Studies have been done that used morphological characteristics to distinguish between subspecies (Schaefer and Cavender 1986; Matthews 1987). Strictly genetic analyses were done to determine if a distinct difference existed between populations (Northcote et al. 1970) and subspecies (Echelle et al. 1975). A few studies combined both morphological and genetic analyses to distinguish between populations (Currens et al. 1990; Sada et al. 1995; Watts et al. 1995) and species (Mayden 1988a). I will use the combined approach because I agree with Wheeler (1993) who stated that “Without the results of both population genetics and systematics, conservation efforts have little hope of success.” Both components of change are necessary to understand intraspecific variation.

## OBJECTIVES

Determine the role that habitat perturbation played in shaping a species.

Determine if dams and impoundments caused genetic isolation between populations.

Determine if stamina was related to body shape and affected by flow velocity.

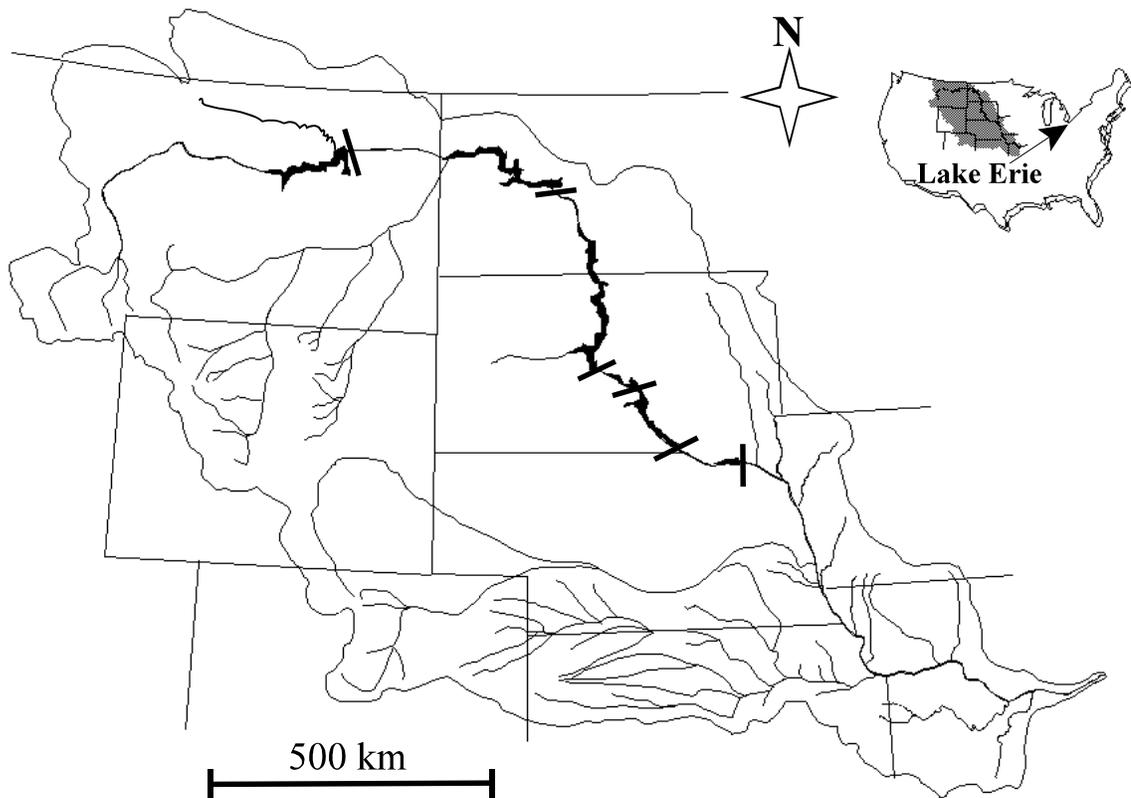
## RESEARCH HYPOTHESES

- Emerald shiner populations separated by dams show evidence of uni-directional and graded genetic divergence.
- Meristic counts for emerald shiners are related to habitat conditions more than a natural latitudinal cline.
- Body shape and physical characteristics of emerald shiners vary in response to habitat changes.
- Populations of emerald shiners living in naturally riverine, channelized, and impounded segments of the river have swimming stammas directly related to the mean flow of their habitat.

## METHODS

### Collection of Fish

Emerald shiners were collected from two different continental watersheds. Fish from the Missouri River were from sections of the river that span from Great Falls, Montana to St. Louis, Missouri (Figure 3). These included non-impacted, inter-reservoir, reservoirs, regulated unchannelized, and regulated channelized river sections. Fish from Lake Erie near Toledo, Ohio were used as an outgroup. Fish were collected using seines,



**Figure 3.** Location of the Missouri River watershed and a Lake Erie sample site. Lines perpendicular to the Missouri River show the location of the six mainstem dams. Channelization begins at the South Dakota, Nebraska, and Iowa tri-state border.

electrofishing, and benthic trawls. Once collected, the fish were preserved in 4% formalin if they were used for meristic or morphometric analyses. If the fish were used for genetic analyses, they were kept alive as long as possible. If they were dead for more than 30 min prior to cryo-preservation in liquid nitrogen, the fish were discarded or alternatively preserved in formalin. The eye and flesh from the right side of the fish and its liver were each removed and stored separately in cryo-vial microcentrifuge tubes and kept at -80°C or below until they were used in genetic analyses.

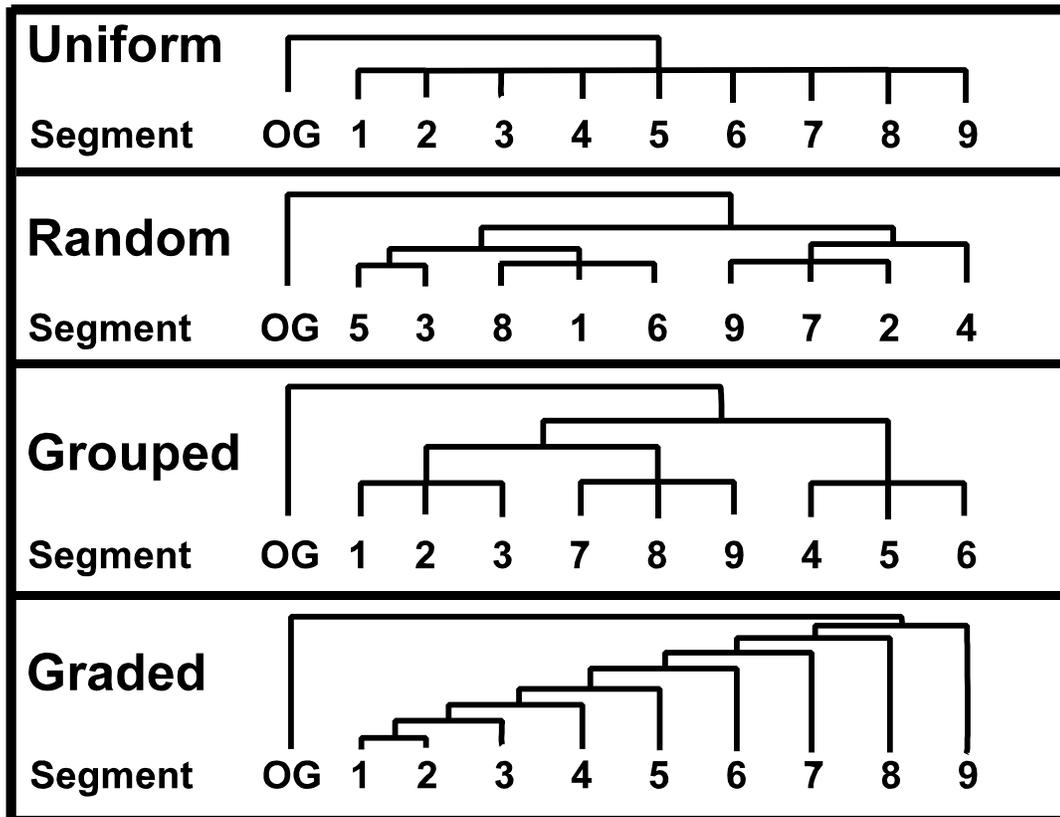
## INTERPRETATION

### Population Structure Responses

The results from the four analyses were designed to support one of five possible outcomes (Figure 4). First, it was possible that the analyses could have contradicted each other in some or all aspects and that relationships between segments of the river were *random*. Sites would be related to other sites in unpredictable and indescribable configurations. For instance, reservoir, Iowa, confluence, and Montana groups may be similar while inter-reservoir, Kansas, Missouri, and North Dakota stocks show similarities. There are no logical geographic or habitat connections between these areas so the relationships seen would be assumed to be the result of random chance.

Second, segments of the river could have displayed a *uniform* configuration throughout the entire river. In this scenario, each segment of the river would be equally dissimilar or alike suggesting that selective pressures were equal throughout the river.

This would suggest that the Missouri River has either 1) a single continuous population



**Figure 4.** The four population structure responses that emerald shiners from nine different sections of the Missouri River and an outgroup (OG) may exhibit.

of emerald shiners, or 2) some type of stock, sub-population, or multiple population structure arising from a formerly continuous, single population that was not identifiable through these character analyses.

Third, segments of the river could have displayed a *grouped* configuration.

Certain characteristics would be shared by several segments of the river that also shared geographic or habitat similarities. For example, Montana, North Dakota, Iowa, Kansas, and Missouri might have exhibited similar characteristics that South Dakota and the reservoirs did not show. This would suggest some type of reservoir and inter-reservoir effect that differentiates those areas from the rest of the river. Similarly, Montana and the Yellowstone River could be grouped, all the reservoirs could be grouped, all the inter-reservoir segments could be grouped, and the lower channelized areas could be grouped. This type of configuration would easily be explained by habitat and local selective pressure differences.

Fourth, a *graded* or *cline* configuration could exist between the segments. This type of arrangement would be the result of individuals that moved between populations in a down-river direction only. As individuals emigrated, they would take characteristics of their former stock with them to their adjacent down-river neighboring stock. This progression would continue allowing characteristics of the upper-most stock to be distributed to all other down-river stocks. Each stock down-river from the upper-most stock would then be able to distribute individuals to all stocks down-river from it, but not to any stocks above its range. This type of character transference could show either no visible response if characteristics were not variable among stocks to begin with or a narrow variation in traits in the upper most stock and a wide range of traits in the lower most stock.

Fifth, a *combination* of the previous four configurations could be revealed. A combination response would be seen if the meristic comparisons showed a random

response, the morphometric comparisons showed a grouped response, and the genetic analyses showed a graded response. A single response could also be shared by two of the three analyses. These combinations all suggest several complicated population configuration scenarios, but all share the underlying theme that characteristics are not all affected equally by the same selective pressures.

### Implications

The types of responses seen suggest the type of population structure possessed by Missouri River emerald shiners and likely, many other species as well. Repeated, absent, and combinations of responses all imply different types of processes that are forming and maintaining the population structure of the entire river. Random or uniform responses mean that either 1) the entire river contains a single continuous population with interbreeding among all segments, 2) the river contains distinctive stocks, but the selective pressures among the river segments are not great enough to cause any changes in the different stocks, or 3) the river contains distinctive stocks, but they have not had enough time to significantly diverge and show character differences. Of these three, the third seems most probable explanation.

A grouped response can only mean that habitat is the deterministic force. Habitat creates different selective pressures causing stocks in similar habitats to exhibit similar characteristics. This response is likely only possible in meristic and morphometric comparisons. If a grouped response is seen in the genetic analyses, it would suggest the highly unlikely scenario that either habitat conditions cause specific mutations or that

individuals passed through a series of river segments without breeding and only started breeding again once they found a similar type of segment further down-river.

A graded response would only support the hypothesis of uni-directional character transferral. Finding a completely step-wise progression of characteristic variability from one end of the river to the other is highly unlikely, but the step-wise trend may appear with some of the steps missing. This type of response supports gene flow as the deterministic force causing the changes or variability displayed.

If the meristic, morphometric, and genetic analyses all show different responses, it means that each analysis must be explained separately and that the three types of characteristic measurements are not acted upon equally by the deterministic forces causing the changes. Each type of characteristic would be then shown to vary independently from the others and respond differently in rate and magnitude to the same conditions. Any combination of responses from the meristic, morphometric, and genetic analyses determines how emerald shiners and probably many other species are related among river segments and helps in understanding how to view the population structure of species throughout the entire river.

## APPLICATIONS

The results of this study are important to scientists and resource managers. Scientifically, this study provides more information about the rate and degree of intraspecific change that results when groups are isolated and subjected to new and altered habitat conditions. Systematics studies have often examined differences between species and tried to determine the cause and time of their divergence. I know the cause and time of what may be the event responsible for the preliminary steps of divergence for emerald shiners. Using that information, I investigated the rate and magnitude of early changes that can begin the process of species divergence. The results also show whether different characteristics change at different rates. Although the correlations, causes, and results of this study are species specific and not necessarily transferrable to other species, the idea of the inter-relationship between meristic, morphometric, and genetic variables and the processes involved are conceptual and can be applied to other species within similar contexts.

Several of the concepts I investigated were cited as important areas of research or “Intellectual Frontiers” for the “Sustainable Biosphere Initiative” of the Ecological Society of America (Lubchenco et al. 1991). My research was directly related to six of their twelve research priorities listed in italics below.

- *“What are the patterns of diversity in nature, and what are their critical ecological and evolutionary determinants?”*

Lubchenco et al. (1991) stated that with the rise of molecular techniques and landscape-level habitat assessment techniques that the relationships

between habitat and organismal change and diversity could be more thoroughly understood. My research used both molecular genetics and landscape-level habitat assessment to measure the variation in both genotype and phenotype of emerald shiners. The results of the two approaches were then used to infer the influence of abiotic conditions on biotic processes.

- *“How do morphological, physiological and behavioral traits of organisms interact?”*

The authors stated that advances in technology now permit the detailed evaluation of morphological variation and provide new insight into the area of eco-morphology. This is exactly what chapter 4 of this dissertation investigates. I used digital image processing and multivariate statistics to identify and discriminate among specific morphometric forms of a species. The results were then applied to known habitat alterations in an attempt to correlate structure and function with local habitat differences.

- *“How plastic are the morphology, physiology, and behavior of organisms in the face of environmental stresses? What are organisms’ proximal limitations?”*

*“Analysis of plasticity is critical to understanding the capacity of organisms to respond to anthropogenic changes and predicting whether environmental changes will cause genetic shifts within populations and taxonomic shifts within communities.”* The intraspecific variation, alluded to in the title of this dissertation, refers to phenotypic plasticity and its

genetic constraints. My research focused on how anthropogenic changes to habitat might push a population to its plastic extremes and how genetic architecture might either actively subdue those changes through selection, allow plasticity as an adaptation, or be altered through drift when fragmented.

- *“What factors explain the life history adaptations of organisms? What are the population-level consequences of these adaptations?”*

Here, Lubchenco et al. (1991) stated that anthropogenic alterations to habitat have provided new motivation to understanding life histories, the phenomena that influence and determine them, and their adaptability to change. Although I did not focus on life histories per se, their changes in response to altered habitat likely affected the morphometric, meristic, and especially the performance sections of my research. For instance, the obvious diet and behavioral differences between reservoir and riverine groups of emerald shiners may have helped to hasten phenotypic shifts.

- *“How does fragmentation of the landscape affect the spread and persistence of populations?”*

*“Natural and human-induced patterns of disturbance interact with species’ traits and interspecific relationships to affect the patterns of spread, persistence, and abundance of species.”* The detailed trait analyses that I conducted were specifically designed to determine the magnitude of phenotypic change produced by selective pressures

associated with habitat fragmentation. Spread, persistence, and abundance are population responses that depend on fitness and reproduction. If fitness and reproduction can be maintained through phenotypic plasticity, then the population responses may be left unaffected.

- *“What are the consequences of environmental variability, including natural and anthropogenic disturbance, for individuals, populations, or communities?”*

Environmental variability can produce long term stability within a population, species, or community. However, quantifying the relationship between habitat variability and biotic stability is extremely difficult. My research involved a unique situation where temporal environmental variability decreased, while spatial environmental variability increased dramatically in the Missouri River. These circumstances allowed me to study a stable population that was adapted to temporal variability, but was fragmented into temporally unvariable, yet very different habitat types. This provided a situation where I could actually determine quantitatively whether the species 1) had an inherent high tolerance of diverse habitat types and adjusted only behaviorally, or 2) whether its adaptability was the result of an amalgamation of historically variable phenotypes, hidden in its genetic architecture, only to be revealed when temporal variability ceased among spatially variable habitats.

The relevance of my research to needs of the field of ecology, and the sub-discipline of evolutionary ecology, seem substantiated by the previous list of research priorities presented by the leadership of the field. My research nor that of any one researcher could hope to fully answer any of those six “*Intellectual Frontiers*”. However, I believe that my studies have contributed information to the questions asked and provided data that shows how one species, under unique conditions, reacts and responds to changes in the environment.

From this study, managers were provided with several important facts about the population biology of a species native to the entire Missouri River. Genetically, we learned whether the impoundments and habitat alterations of the Missouri River successfully isolated sub-populations into distinct new populations. Morphometrically and meristically, we learned whether the habitat alterations caused selection for distinctly different characteristics in different habitats. By measuring stamina through performance, I determined whether habitat alterations concomitantly altered selective pressures, resulting in populations with stamias correlated to their habitat.

The results of my research should be of great interest to United States Army Corps of Engineers and the other agencies that manage the Missouri and other rivers as signifying a type of tolerance boundary or limit. As explained earlier, of the species currently and historically residing in the Missouri River, emerald shiners can be thought of as one of the most generalist and adaptable species. However, the impact of river alterations has likely affected the majority of the other Missouri River fishes in similar ways. The other more sensitive and specialized species are likely experiencing more

stress. Determining that specific habitat types have affected phenotypic change in emerald shiners may indicate that more serious changes in abundance, persistence, reproduction, and other population attributes may be afflicting other more sensitive species. In emerald shiners for instance, a change in a meristic count caused by loss of warm-water spawning habitat around sand bars may equate to a decrease in reproductive success among species that depend on that type of habitat for spawning. The data from my study can only be directly applied to understanding emerald shiners, but the changes and shifts seen in emerald shiners are a function of their habitat. That habitat is common to all Missouri River species and will affect some type of change in those species as well.

Perhaps the most important management implication is that because of river alteration, a single species that once was tolerant of a wide range of habitat conditions may have become specialized into several distinctly different and identifiable groups constituting new sub-populations. These new sub-populations can no longer be managed collectively, they must be evaluated separately. Conspecifics in adjacent river segments may require very different habitat conditions for their persistence. Populations that are more specialized tolerate a narrower range of habitat conditions for survival. Thus, a return to historic Missouri River hydrology may result in the decline of such specialized populations because it is easier for a generalist to become specialized than it is for a specialist to become generalized. This type of ecological guild shift can apply to species other than just emerald shiners. Any species, regardless of its current relative generalist or specialist classification, can become more specialized when the variability of its habitat is lost; however, when variability is increased, a specialist will be unlikely to diversify to

the mode of a generalist. The present alterations may be creating an environment where species will depend on managers maintaining the highly regulated conditions and actually suffer if exposed to the former highly variable river conditions. If the river is engineered and managed at the local level, then species may have to be managed on the same scale.

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## Chapter 2

Genetic Population Structure of Emerald Shiners (Cyprinidae: *Notropis atherinoides*)  
in the Missouri and Yellowstone Rivers

## ABSTRACT

I used allozymes to determine if dams in the Missouri River basin have created fragmented, adjacent sub-populations of *Notropis atherinoides*. Three loci significantly differed between two of five sites. Confidence intervals for  $\theta$  (a multi-locus estimate of gene fixation) overlapped among all site groupings refuting distinct sub-population status. Genetic distance estimates indicated all mainstem Missouri River sites were more similar to each other than any were to the Yellowstone River. The mainstem Missouri River remains effectively panmictic, but the Yellowstone River may represent an evolutionary unit.

## INTRODUCTION

The historic population of emerald shiners (Cyprinidae: *Notropis atherinoides*) in the Missouri River was potentially interbreeding, with no physical barriers to impede bi-directional migration in the system. Historic genetic population structure within the Missouri River basin is unknown, but assumed to be homogenous based on current distribution and ecology of the species. Emerald shiners are annual spawners and live an average of two to three years with four being the oldest recorded (Fuchs 1967). This ensures frequent mating and relatively short generations. Unlike many riverine species, emerald shiners are not migratory, spawn in open water, and produce planktonic eggs (Flittner 1964) thereby preventing the formation of distinct breeding groups. Thus any population structure observed in Missouri River emerald shiners is likely due to the natural patchiness of spawning habitat (Hartl 1980) and perhaps isolation of sub-populations via dams.

Emerald shiners inhabit 3500 km of the Missouri River from its mouth to the natural barrier at Great Falls, Montana. This portion of the river has been fragmented by six dams (22-75 m high) constructed from 1937 to 1963 with impoundments ranging from 100 million to 23.3 billion m<sup>3</sup> in storage capacity (Schmulbach et al. 1992; Becker and Gorton 1995). The dams prevent upstream migration, but allow entrainment downstream (Walburg 1971). As a result of dam construction, 1095 km of riverine habitat became lacustrine. The lower 1212 km of the river were channelized, thereby increasing depth and velocity and homogenizing habitat (Hesse et al. 1989; Schmulbach et al. 1992). The result was a discontinuous concatenation of diverse habitats with fish

communities potentially confined to a single river reach. Movement outside the enclosed reaches would only occur by passing through the dams to the next downstream reach.

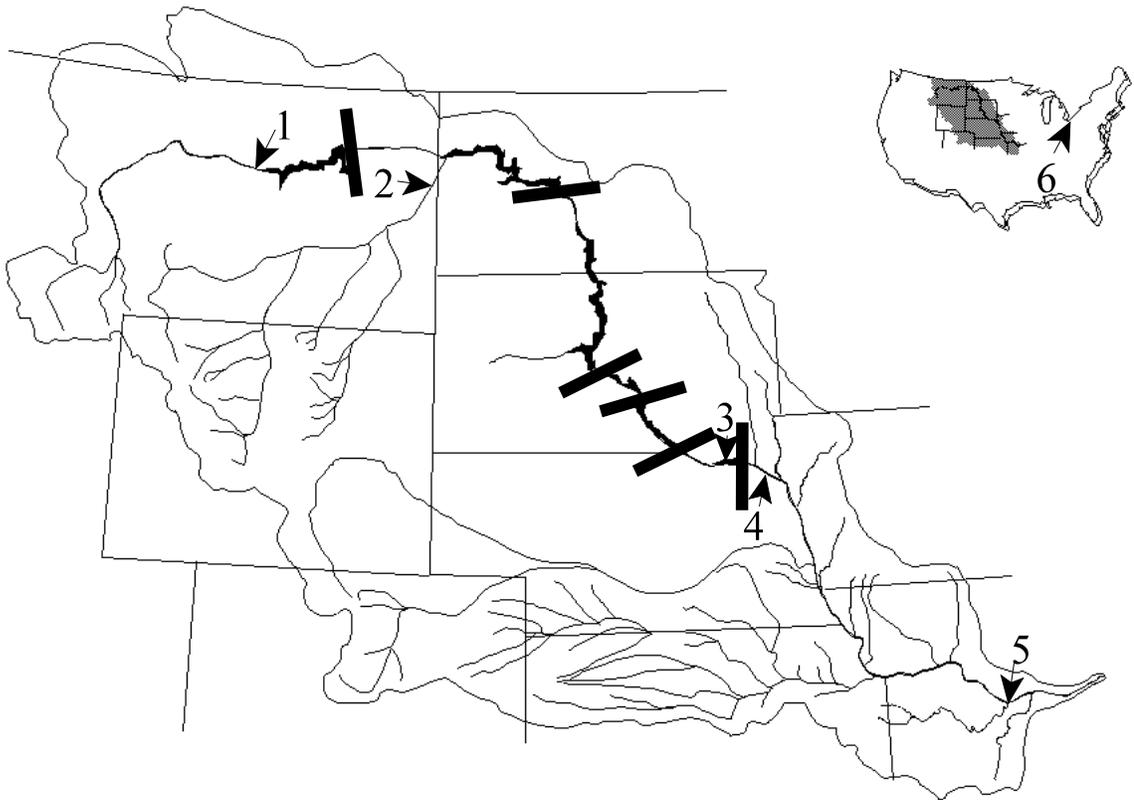
Few studies on the genetic population structure of non-anadromous species in large rivers have been done. Phelps and Allendorf (1983) studied the genetics and hybridization of the genus *Scaphirhynchus* and Epifanio et al. (1996) studied the geographic genetic variation in *Polyodon spathula* throughout the Mississippi River basin, but because sturgeon and paddlefish are migratory, long-lived species, their genetic population structure is not comparable to that of emerald shiners. Lu et al. (1997) found genetically distinct populations of carps in the Yangtze River of China, but attributed the differences to the migration of groups of carp to consistent, specific, and separate breeding grounds. Watts et al. (1995) found no genetic differentiation in *Galaxias occidentalis* between sites separated by dams within each of two Australian rivers, but their sites were less than 50 km apart. Guinand et al. (1996) identified distinct genetic differentiation in parts of the Rhône River and attributed this to a disruption in gene flow caused by dams and regulated hydrology. Dams plus channel alterations also produce new and differing selective pressures that may enhance intraspecific divergence (Williams and Wilde 1981; Rutledge et al. 1990). That degree of divergence may be amplified because riverine species tend to be more heterozygous and genetically variable than their lacustrine counterparts (Wong and McAndrew 1994) thus enabling more highly differentiated adaptation among new habitats.

The purpose of this study was to determine whether genetically distinct sub-populations of emerald shiners exist in the Missouri River. The time since potential

isolation (40-60 years) and number of generations (15-30) would typically be thought as too short a time to detect differences, yet Hendry et al. (1998) used allozymes to identify two distinct populations of salmon that were part of a common parent population only 9 to 14 generations ago. Genetic drift, potentially accelerated by habitat alteration, coupled with fragmentation by dams, provide conditions favorable to the rise of genetically distinct sub-populations. If genetic population divergence has begun in response to gene flow impedance by the dams, then river resource managers should be aware that they may be producing distinct genetic stocks that were once historically homogenous (Lu et al. 1997).

## MATERIALS AND METHODS

Emerald shiners were collected from four sites on the Missouri River, one site in the Yellowstone River, and one site in Lake Erie (Figure 1). The Montana site was located in the Charles M. Russell National Wildlife Refuge about 2 km upstream from the highway 191 bridge. The Yellowstone River site was about 53 river kilometers upstream from its confluence with the Missouri River located about 2 km upstream of the highway 23 bridge near Sidney, Montana. Lewis and Clark Lake fish were sampled off the South Dakota shoreline about 7 km east of Springfield, South Dakota. The Vermillion, South Dakota site was adjacent to the Clay County boat launch. The Missouri site was at the confluence of the Osage and Missouri rivers. Emerald shiners were collected off the Osage River side of the dike, about 50-100 m from the confluence. The Lake Erie site was located near Toledo, Ohio at the mouth of the Maumee River. Fish were collected by



**Figure 1.** Location of the 6 sites: 1) Missouri River, Montana (river km 3101), 2) Yellowstone River, 3) Lewis and Clark Lake (river km 1330), 4) Missouri River near Vermillion, South Dakota (river km 1256), 5) Osage River and Missouri River confluence, Missouri (river km 209) and Lake Erie (Toledo, Ohio). The 6 lines across the Missouri River show the locations of dams.

electrofishing or seining and euthanized immediately. Liver, eye, and muscle tissue samples were taken from each individual and stored in liquid nitrogen. Gel electrophoresis was performed for 28 different loci using the methods described by May (1992) and appropriate gel buffers (Table 1).

Expected Hardy-Weinberg genotype frequencies were calculated using Levene's (1949) correction for small sample sizes at polymorphic loci from all sites using likelihood ratio tests for homogeneity.  $N_e m$  (the effective number of migrants exchanged per generation) and 95% confidence intervals of  $\theta$ , a multilocus estimate of gene fixation (Weir and Cockerham 1984), were calculated for eight groupings based on geographic proximities. An unweighted pair group method of averages (UPGMA) phenogram was constructed from Nei's (1978) unbiased genetic distances to show similarity among groups. Likelihood ratio tests of homogeneity among groups, based on allele frequencies, were performed on the Missouri River basin groups using two-way contingency tables. These tests identify specific loci that have significantly different gene frequencies between groups and may indicate population subdivision.

**Table 1.** Loci, enzyme systems, number of loci, tissues used, Enzyme Commission Numbers (ECN), and buffers used to survey emerald shiners from the Missouri River.

| Locus  | Enzyme                                   | Loci ( <i>n</i> ) | Tissue <sup>1</sup> | ECN       | Buffer <sup>2</sup> |
|--------|--|-------------------|---------------------|-----------|---------------------|
| sAAT   | Aspartate aminotransferase               | 1                 | M                   | 2.6.1.1   | R                   |
| CK     | Creatine kinase                          | 1                 | M                   | 2.7.3.2   | R                   |
| EST    | Fluorescent esterase                     | 3                 | L                   | 3.1.1.-   | TC                  |
| GAPDH  | Glyceraldehyde-3-phosphate dehydrogenase | 2                 | M                   | 1.2.1.12  | C                   |
| GPI    | Glucose-6-phosphate isomerase            | 4                 | M                   | 5.3.1.9   | R                   |
| G6PDH  | Glucose-6-phosphate 1-dehydrogenase      | 1                 | L                   | 1.1.1.49  | R                   |
| HBDH   | 3-Hydroxybuterate dehydrogenase          | 1                 | M                   | 1.1.1.30  | TC                  |
| IDH    | Isocitrate dehydrogenase                 | 1                 | M                   | 1.1.1.42  | TC                  |
| sMDH   | NAD Malate dehydrogenase                 | 2                 | M                   | 1.1.1.37  | 4                   |
| MPI    | Mannose-6-phosphate isomerase            | 2                 | M                   | 5.3.1.8   | 4                   |
| PEPA   | Peptidase-glycyl-leucine                 | 1                 | L                   | 3.4.-.-   | R                   |
| PEP LA | Peptidase-leucyl-alanine                 | 2                 | L                   | 3.4.-.-   | R                   |
| PGDH   | Phosphogluconate dehydrogenase           | 1                 | L                   | 1.1.1.44  | TC                  |
| PGM    | Phosphoglucomutase                       | 2                 | M                   | 5.4.2.2   | TC                  |
| PRO    | General (unidentified) protein           | 2                 | M                   | -.-.-.-   | C                   |
| sSOD   | Superoxide dismutase                     | 1                 | L                   | 1.1.15.1  | R                   |
| XDH    | Xanthine dehydrogenase                   | 1                 | M                   | 1.1.1.204 | C                   |

<sup>1</sup> M = Muscle and L = liver.

<sup>2</sup> R - Ridgeway, G.S., S.W. Sherburne, and R.D. Lewis. 1970. Polymorphism in the esterases of Atlantic herring. Transactions of the American Fisheries Society 99: 146-151.

TC - Whitt, G.S. 1970. Developmental genetics of the lactate dehydrogenase isozyme of fish. Journal of Experimental Zoology 175:1-36.

- C - Clayton, J. W., and D. N. Tretiak. 1972. Aminocitrate buffers for pH control in starch gel electrophoresis. *Journal of the Fisheries Research Board of Canada* 29:1169-1172.
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## RESULTS

Of the 28 loci surveyed, 14 different polymorphic loci were found: five at the Montana site, seven in the Yellowstone River, five in Lewis and Clark Lake, three at the Vermillion, SD site, six at the Osage River and Missouri River confluence, and seven in Lake Erie (Table 2). Of 34 likelihood ratio tests for homogeneity, only EST-3 in the Montana group and PEPLA-2 in the Missouri group were found to statistically deviate from Hardy-Weinberg equilibrium as heterozygote deficient. Given that 1 in 20 comparisons at each site is expected to deviate from Hardy-Weinberg expectations due to chance, these results were not considered further. Mean heterozygosity was low overall with the highest levels seen in the Yellowstone River and Lake Erie groups (Table 2). Unique alleles were found in the Montana group at the MDH-1 and GPI-1 loci, in the Yellowstone River group at the MDH-2 and PEPLA-1 loci, and in the Lake Erie group at the GAPDH-2, GPI-1, and EST-3 loci (Table 2).

**Table 2.** Allelic frequencies of polymorphic loci for emerald shiners collected from the Missouri River, Montana (MT), Yellowstone River (YR), Lewis and Clark Lake (LC), Missouri River near Vermillion, South Dakota (SD), the Osage River and Missouri River confluence (MO), and Lake Erie (LE). B-E = allele types; (N) = sample size.

| Loci    | Alleles | MT    | YR    | LC    | SD    | MO    | LE    |
|---------|---------|-------|-------|-------|-------|-------|-------|
| MPI-1   | (N)     | (30)  | (27)  | (30)  | (29)  | (22)  | (29)  |
|         | B       |       |       | 0.017 |       | 0.132 |       |
|         | C       | 1.000 | 1.000 | 0.966 | 0.983 | 0.842 | 1.000 |
|         | D       |       |       | 0.017 | 0.017 | 0.026 |       |
| sMDH-1  | (N)     | (30)  | (29)  | (30)  | (29)  | (30)  | (29)  |
|         | C       | 0.983 | 1.000 | 1.000 | 1.000 | 1.000 | 1.000 |
|         | D       | 0.017 |       |       |       |       |       |
| sMDH-2  | (N)     | (30)  | (30)  | (30)  | (29)  | (28)  | (29)  |
|         | B       |       | 0.017 |       |       |       |       |
|         | C       | 1.000 | 0.914 | 1.000 | 1.000 | 1.000 | 1.000 |
|         | D       |       | 0.069 |       |       |       |       |
| GAPDH-2 | (N)     | (30)  | (30)  | (30)  | (29)  | (27)  | (30)  |
|         | C       | 1.000 | 1.000 | 1.000 | 1.000 | 1.000 | 0.983 |
|         | D       |       |       |       |       |       | 0.017 |
| GPI-1   | (N)     | (30)  | (28)  | (30)  | (29)  | (27)  | (30)  |
|         | B       | 0.017 |       |       |       |       |       |
|         | C       | 0.933 | 1.000 | 1.000 | 1.000 | 1.000 | 0.917 |
|         | D       | 0.050 |       |       |       |       | 0.050 |
|         | E       |       |       |       |       |       | 0.033 |
| GPI-4   | (N)     | (30)  | (28)  | (30)  | (29)  | (27)  | (20)  |
|         | B       |       |       |       |       | 0.021 | 0.025 |
|         | C       | 1.000 | 1.000 | 1.000 | 1.000 | 0.971 | 0.975 |
| PGM-1   | (N)     | (29)  | (30)  | (30)  | (29)  | (7)   | (30)  |
|         | C       | 0.948 | 0.983 | 0.983 | 1.000 | 1.000 | 1.000 |
|         | D       | 0.052 | 0.017 | 0.017 |       |       |       |
| PGDH    | (N)     | (26)  | (25)  | (20)  | (17)  | (25)  | (13)  |
|         | B       |       |       |       |       | 0.025 | 0.385 |
|         | C       | 1.000 | 1.000 | 1.000 | 1.000 | 0.975 | 0.615 |
| EST-1   | (N)     | (17)  | (7)   | (29)  | (29)  | (10)  | (28)  |
|         | B       |       | 0.071 | 0.035 | 0.017 |       |       |
|         | C       | 1.000 | 0.929 | 0.965 | 0.983 | 1.000 | 1.000 |
| EST-2   | (N)     | (24)  | (30)  | (15)  | (27)  | (9)   | (8)   |
|         | B       | 0.125 | 0.117 | 0.233 | 0.192 | 0.167 | 0.250 |
|         | C       | 0.875 | 0.883 | 0.767 | 0.808 | 0.833 | 0.750 |

*continued on following page*

**Table 2. Continued**

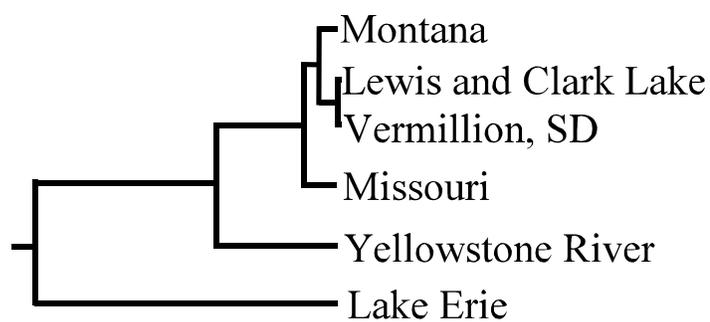
| Loci                           | Alleles | MT    | YR    | LC    | SD    | MO    | LE    |
|--------------------------------|---------|-------|-------|-------|-------|-------|-------|
| EST-3                          | (N)     | (28)  | (30)  | (29)  | (29)  | (26)  | (25)  |
|                                | B       | 0.054 | 0.267 | 0.086 | 0.017 | 0.024 | 0.060 |
|                                | C       | 0.946 | 0.733 | 0.914 | 0.983 | 0.976 | 0.820 |
|                                | D       |       |       |       |       |       | 0.120 |
| G6PDH                          | (N)     | (17)  | (24)  | (9)   | (10)  | (2)   | (17)  |
|                                | B       |       | 0.083 |       |       |       |       |
|                                | C       | 1.000 | 0.917 | 1.000 | 1.000 | 1.000 | 1.000 |
| PEPLA-1                        | (N)     | (30)  | (30)  | (30)  | (30)  | (11)  | (30)  |
|                                | C       | 1.000 | 0.983 | 1.000 | 1.000 | 1.000 | 1.000 |
|                                | D       |       | 0.017 |       |       |       |       |
| PEPLA-2                        | (N)     | (10)  | (27)  | (17)  | (14)  | (30)  | (30)  |
|                                | B       |       |       |       |       | 0.060 | 0.033 |
|                                | C       | 1.000 | 1.000 | 1.000 | 1.000 | 0.940 | 0.967 |
| Mean heterozygosity            |         | 0.020 | 0.039 | 0.017 | 0.012 | 0.022 | 0.038 |
| Proportion of polymorphic loci |         | 0.179 | 0.250 | 0.179 | 0.143 | 0.214 | 0.250 |

Among all sites,  $N_e m = 3.1$  and  $\theta = 0.061$  (CI = -0.001 - 0.153). Excluding the Lake Erie group gave a  $N_e m = 4.83$  and  $\theta = 0.029$  (CI = -0.004 - 0.077) for the Missouri River Basin. Excluding both the Lake Erie and Yellowstone groups yielded a  $N_e m = 9.93$  and  $\theta = -0.004$  (CI = -0.014 - 0.048) for the mainstem Missouri River. Between Vermillion, SD and the Osage River confluence in Missouri, the longest unimpounded stretch,  $N_e m = 13.610$  and  $\theta = -0.011$  (CI = -0.040 - 0.083). Between Lewis and Clark Lake and Vermillion, SD (the two nearest groups separated by a dam),  $N_e m = 37.220$  and  $\theta = -0.025$  (CI = -0.027 - 0.020). Between the Montana and Yellowstone River groups, separated by a dam,  $N_e m = 6.040$  and  $\theta = 0.063$  (CI = 0.0001 - 0.109). The Montana and Lewis and Clark Lake groups are separated by five dams and have an  $N_e m = 15.850$  and  $\theta = 0.006$  (CI = -0.011 - 0.023). The Yellowstone River and Lewis and Clark Lake groups are separated by four dams and have an  $N_e m = 6.960$  and  $\theta = 0.043$  (CI = -0.007 - 0.070).

Pair-wise unbiased genetic distances (Nei 1978) ranged from 0 to 0.0076 while geographic distances ranged from 74 to 2892 km among sites (Table 3). The UPGMA phenogram, based on these unbiased genetic distances, places the Lake Erie site as most distant. This is consistent with that site being in a separate watershed where individuals were unable to interbreed with individuals from the other five sites. The rest of the genetic distances are also consistent with geographic distances, except for the Yellowstone River site and the Missouri site. All mainstem Missouri River sites are more closely related to each other than to the Yellowstone River site even though it is a shorter geographic distance to several Missouri River sites (Figure 2).

**Table 3.** Distance between sites (river kilometers between Missouri River basin sites and straight line kilometers to the Lake Erie site)[above diagonal] and Nei's (1978) unbiased measure of genetic distance [below diagonal]. Numbers in parentheses indicate the number of dams between these sites.

| Group | MT     | YR      | LC       | SD       | MO       | LE    |
|-------|--------|---------|----------|----------|----------|-------|
| MT    | *****  | 607 (1) | 1771 (5) | 1845 (6) | 2892 (6) | 2090  |
| YR    | 0.0022 | *****   | 1270 (4) | 1345 (5) | 2391 (5) | 1797  |
| LC    | 0.0005 | 0.0018  | *****    | 74 (1)   | 1121 (1) | 1190  |
| SD    | 0.0003 | 0.0027  | 0.0000   | *****    | 1047 (0) | 1127  |
| MO    | 0.0009 | 0.0034  | 0.0006   | 0.0005   | *****    | 815   |
| LE    | 0.0061 | 0.0076  | 0.0055   | 0.0059   | 0.0060   | ***** |



**Figure 2.** Genetic distance phenogram showing relatedness (UPGMA Method) among the six sites for *Notropis atherinoides*.

The Lewis and Clark Lake and Vermillion, SD sites are more closely aligned with the Montana group than their nearer neighbor in Missouri.

Likelihood ratio tests of homogeneity among groups, based on gene frequency, were performed on Missouri River basin sites using two-way contingency tables. The MDH-2 ( $P = 0.038$ ) and EST-3 ( $P < 0.001$ ) loci were responsible for separating the Yellowstone River site from all other Missouri River Basin groups as a distinct population. The MPI-1 locus ( $P = 0.008$ ) identified the Missouri site as distinct from the other mainstem Missouri River groups.

## DISCUSSION

Emerald shiners are effectively panmictic from Montana to South Dakota in the mainstem Missouri River even though there are six dams within this river reach. There are no dams in the 1047 km reach separating the Vermillion, SD and Missouri sites, but one locus identified the Missouri site as distinct from the rest of the river. The Yellowstone River group showed an unexpected divergence. It was significantly different from the Missouri River groups at two loci, but estimates of  $\theta$  and its confidence intervals indicate that genetically distinct populations do not exist among any of the sites sampled. This may not necessarily mean that distinct populations or sub-populations do not exist or function independently among the sites, but does suggest that genetic differentiation among them is not sufficient to warrant designation as genetically distinct populations. I am cautious in my interpretation of the negative data, knowing that other loci may reveal more pronounced population differences (Utter et al. 1992). The

values of  $\theta$  follow a trend of decreasing means and narrowing confidence intervals going from all sites, to all Missouri River Basin sites, to mainstem Missouri River sites. This pattern is consistent with the genetic distance (Table 3) and homogeneity test results.

Genetic distances indicate a 2-4 times greater degree of distance between the Yellowstone River and all other mainstem Missouri River sites than the mainstem Missouri River sites show among one another. Additionally, mean heterozygosity is highest at the Yellowstone River site. These results may warrant the designation of the Yellowstone River as a genetically distinct sub-population or evolutionary unit (National Research Council 1995). The 53 km distance from the Yellowstone River site to the confluence with the mainstem Missouri River should not cause isolation-by-distance considering the panmixia suggested among mainstem Missouri River sites separated by 1845 km. However, the Yellowstone River divergence is consistent with a study by Baer (1998) who found no genetic differentiation in *Heterandria formosa* within a fork of the St. John's River, but significant differentiation between fish from sites in adjacent forks. It is possible that environmental factors prevent the transfer of individuals between the Yellowstone River and the Missouri River. Morán et al. (1995) found that species can be more genetically related to geographically distant conspecific populations than to adjacent populations because of behavioral and environmental differences. Mean depth and velocity are lower while mean temperature, turbidity, and substrate coarseness are substantially higher in the Yellowstone River than in the adjacent Missouri River (Young et al. 1997). These differences are attributable to the regulated hydrology of the Missouri River versus the natural hydrology of the Yellowstone River. These habitat variables

may promote reproductive isolation through life history and ecology differences between the adjacent sites. Verspoor et al. (1991) also identified variation in physical habitat among tributaries as a selective force responsible for local adaptation and differentiation of populations within a basin. The relative roles of genetic drift and selection in this differentiation are impossible to ascertain with the current genetic data, but I believe that genetic differentiation between Yellowstone River and mainstem Missouri River populations has resulted from isolation via conflicting environmental stresses (Endler 1977; Slatkin 1985).

There are two possible explanations for the population homogeneity over the length of the mainstem Missouri River. First, the current uniform genetic population structure may be remnant of the Missouri River before alteration. Second, there is enough downstream migration and passage through the dams to negate isolation caused by the mainstem dams and reservoirs. If so, then the small anomaly between the Missouri site and the other mainstem sites may be representative of an allele originating in the lower river that has been unable to be passed upstream.

Emerald shiners are not representative of the entire fish community of the Missouri River. Other non-migratory, short-lived, small bodied species like emerald shiners (*Notropis spp.*, *Cyprinella spp.*, *Pimephales spp.*) should also be among those species that have the greatest potential for genetic divergence throughout the basin. However, I found little evidence of any divergence. It is possible that the Missouri River is currently in a transitional period and approaching equilibrium between gene flow and drift among the discontinuous habitats. That equilibrium may take hundreds of years

(Allendorf and Phelps 1981; Chakraborty and Leimar 1987a), yet only 40 to 60 years have passed since dam construction began affecting the gene flow of Missouri River emerald shiners.

This study has shown that there is little evidence of genetically distinct sub-populations of emerald shiners in the mainstem of the Missouri River. This information indicates that even in a species with characteristics favoring the fastest potential rate of genetic divergence, this riverine population remains effectively panmictic. I can only speculate that over the course of time, if the current conditions continue, genetically distinct sub-populations will eventually emerge in this setting. If these emerald shiner data are also representative of confamilial unstudied species of concern (e.g. *Macrhybopsis gelida* and *Macrhybopsis meeki*), then decisions about their conservation might also be made with little regard for the presence of distinct sub-populations. Identifying the time required for species to undergo population restructuring in specific circumstances will help us gain an understanding of the importance of various factors and their role in population substructure formation (Chakraborty and Leimar 1987b). The ability to define and predict changing genetic population structure as it relates to anthropogenic habitat fragmentation, also provides ecosystem managers with the information needed to effectively conserve species in the future.

## ACKNOWLEDGMENTS

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## Chapter 3

## Meristic Variation in Response to Habitat Alteration

## ABSTRACT

I used meristic counts of *Notropis atherinoides* to detect whether abrupt habitat changes in the Missouri River were sufficient to disrupt the natural clines typically seen across latitude and temperature gradients. Counts of dorsal, anal, pectoral, and pelvic fin rays and vertebrae showed that only pectoral fin rays (9-14) and vertebrae (35-43) differed among sites ( $P < 0.001$ ). Ray numbers increased in and near reservoirs of the Middle Missouri River and decreased both north and south of the reservoirs. Latitude and water temperature, measured as degree days, both showed a peaking parabolic relationship with pectoral fin rays while turbidity had an inverse linear relationship with ray number. Vertebrae showed a linear relationship with latitude and growth, increasing toward the more northern sites, indicating that the two meristic features varied independently. Mean vertebrae counts appeared to be unaffected by habitat alteration, but river channelization in the lower third of the Missouri River may be responsible for the disruption in the natural pectoral fin ray cline. The loss of sandbar habitat used for spawning and rearing in the Lower Missouri River may be suppressing what would naturally be an increasing cline in pectoral fin rays with water temperature and latitude.

## INTRODUCTION

The meristic characters of fishes have long been known to vary in response to environmental gradients (Jordan 1891; Hubbs 1922). Water temperature, light, water chemistry, and simple isolation can produce intraspecific meristic variation (Ali 1962; Fowler 1970; Weldon 1992). Changes in these environmental conditions usually vary in a gradual cline across latitude or other environmental gradients (Endler 1977). However, there are situations where local conditions vary greatly from one site to another. This is the case in the Missouri River where 1,095 km of historically riverine habitat has been impounded in six reservoirs. Of the remaining 2405 km riverine habitat, the 1212 km preceding the mouth have been channelized thereby increasing depth and velocity and homogenizing habitat (Hesse et al. 1989; Schmulbach et al. 1992). Prior to alteration, the Missouri River functioned and varied according to the general pattern of the river continuum concept (Vannote et al. 1980). After alteration, patterns and processes were abruptly changed so that the Missouri River now follows the serial discontinuity concept (Ward and Stanford 1983; Stanford et al. 1988). Hydrology, water temperature, turbidity, and water chemistry now vary in response to dam regulation and channel alteration forming a series of adjacent, but abruptly fragmented habitats. These disjunct physicochemical differences in habitat may create a situation where phenotypically plastic species could exhibit distinct characteristics in neighboring habitats.

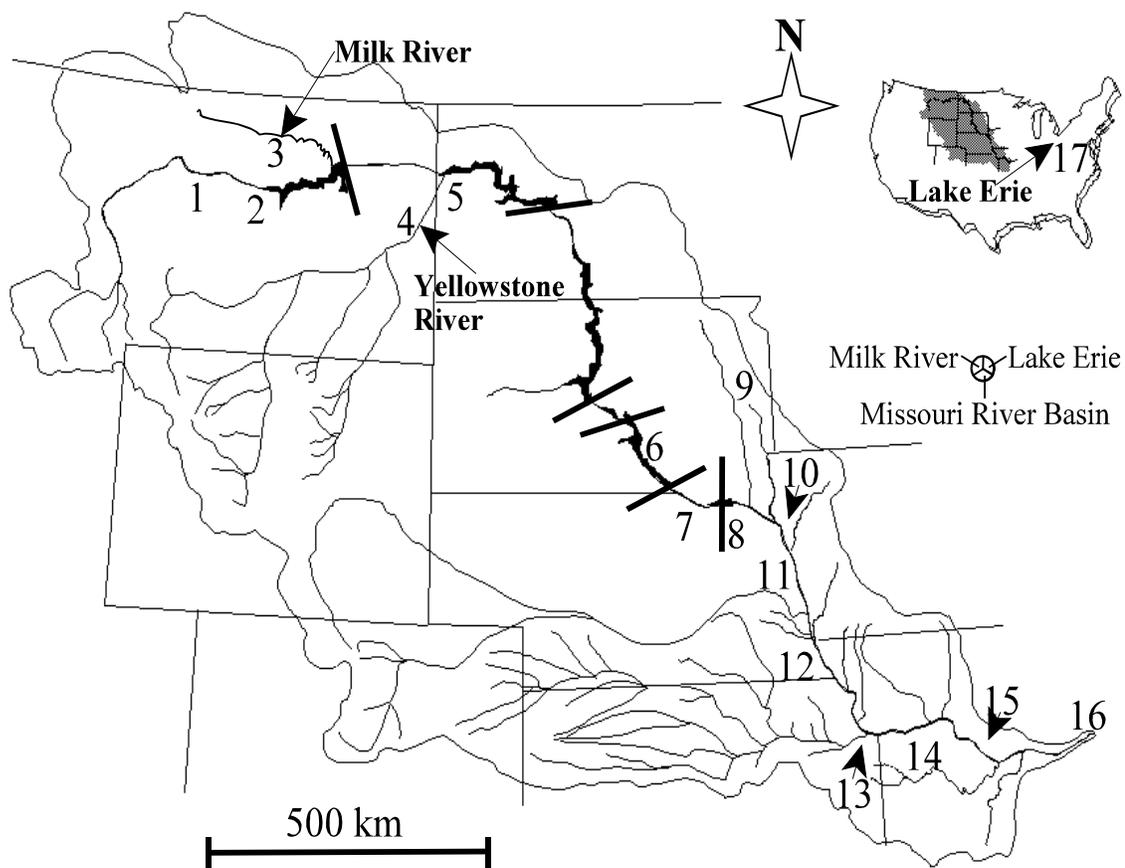
Meristic characters are not solely controlled by environmental factors. Genetic architecture defines the developmental process and determines the array of variable phenotypes or norm of reaction (Schmalhausen 1949). That variability can be species,

character, and population specific. I chose to study emerald shiners (*Notropis atherinoides*) because they have a record of high meristic variability throughout their range (Flittner 1964; Bailey and Allum 1962). Therefore, their potential for meristic differentiation made them an ideal species for determining whether the magnitude of habitat alteration in the Missouri River was sufficient to cause intraspecific meristic variation. The life history of emerald shiners also made them preferable subjects. They begin spawning when water temperature reaches about 24°C and hatch within 32 hours of fertilization (Flittner 1962; Scott and Crossman 1973). This short embryonic period minimizes exposure to wide water temperature shifts and shocks that may influence several aspects of the developmental process (Fowler 1970).

Meristic characters influenced by environmental stressors can be seen within any generation. Unlike genetic differentiation between potential sub-populations, meristic phenotypes can operate independently from gene flow within their norm of reaction. Therefore, environmental stressors may serve to differentiate phenotypically plastic local individuals and reveal intraspecific groupings based on phenotype, regardless of genetic similarity to neighboring conspecifics. Meristic differentiation among groups of fish from different segments of the Missouri River does not necessarily signify the presence of distinct populations, but it may reveal how localized habitat alteration could potentially disrupt natural gradients. This study was conducted to determine whether an historically continuous population that inhabited a gradually changing habitat has become meristically differentiated in response to sudden and disjunct habitat alterations.

## METHODS

Emerald shiners were collected from 14 sites in the Missouri River, the Yellowstone River, the Milk River, and from 1 site in Lake Erie (Figure 1). All fish were fixed in 4% formalin and preserved in 70% ethanol. Five meristic characters were counted on individuals of various size and year-classes within and among sites. Dorsal, anal, right side pectoral, and right side pelvic rays were each counted under a dissecting scope with a lighted background (Strauss and Bond 1990). The fish were then cleared and stained to make vertebral counts (Cailliet et al. 1986). Mean differences among sites



**Figure 1.** Location of the 17 sites in the Missouri River Basin and Lake Erie.

were detected using a Kruskal-Wallis analysis of variance (data not normally distributed) for the four fin ray types. Paired comparisons ( $\alpha=0.05$ ) were made using a nonparametric multiple comparisons test (Zar 1984). Vertebrae data (normally distributed) were analyzed using a one-way analysis of variance and Tukey's HSD paired comparisons test ( $\alpha=0.05$ ) for unequal sample sizes.

Latitude and longitude were recorded at the midpoint of each sample site. Various federal and state agencies provided daily water temperature data from gauging stations near the sites for June and July, 1995-1998. Water temperature data were converted to degree days (sum of all temperatures) for the months of June and July. Turbidity data are highly variable across habitat types, so I used a combination of six representative habitat types for each site and measured daily turbidity, in nephelometric turbidity units (NTU), during a two month period to calculate mean turbidity per site using a YSI-80 turbidimeter. Velocity data were collected in the same format as the turbidity data and combined to provide mean velocity per site using a Marsh-McBirney Flomate velocity meter. I also used otolith aging and length measurements to determine growth rates at sites measured as mm grown per degree day (Braaten 2000). Meristic values were then plotted as a function of latitude, water temperature, turbidity, velocity, and growth rate, both individually and as groups, to identify any correlations.

## RESULTS

The frequency distributions of pectoral rays (Table 1), pelvic rays (Table 2), dorsal rays (Table 3), anal rays (Table 4), and vertebrae (Table 5) showed differences in

variability for each meristic character. Pectoral ray number ranged from nine to fourteen and anal rays ranged from eight to thirteen making them more variable than the range of seven to nine for both pelvic and dorsal rays. Of the four fins, only pectoral ray number was significantly different among sites ( $P < 0.001$ ). Pectoral ray counts also varied widely within and between sites (Figure 2). Although there was high variation within sites, there was a general trend of increased pectoral fin ray number among the lentic and inter-reservoir sites.

The mean number of pectoral fin rays for emerald shiners in and below Middle Missouri River reservoirs (sites 6-8) and in a warm-water tributary (site 9) were higher than for any site in the Upper Missouri River (Figure 2). Sites 10 and 11, the first two sites in the channelized portion of the river, showed a decrease in mean pectoral fin ray number from their immediate up-river neighbors. Site 12, near the mouth of the Platte River, then increased up to the highest mean pectoral fin ray number of any Missouri River Basin site. Site 13, near Kansas City, Missouri, had the second lowest mean ray number. Sites 13-16 in the Lower Missouri River shared lower mean ray numbers that were more similar to the Upper Missouri River rather than the adjacent Middle Missouri River. All Missouri River Basin sites had lower mean pectoral fin ray counts than the Lake Erie site. Middle Missouri River sites (6-8), the warm-water tributary site (9), and the site near the Platte River mouth (12) were not significantly different from the Lake Erie site (17).

**Table 1.** Frequency distribution of pectoral fin ray counts for *Notropis atherinoides* from 16 Missouri River Basin sites and Lake Erie. See Figure 1 for site location.

| Site | Pectoral fin rays |    |    |    |    |    | <i>n</i> | $\bar{x}$ |
|------|-------------------|----|----|----|----|----|----------|-----------|
|      | 9                 | 10 | 11 | 12 | 13 | 14 |          |           |
| 1    |                   |    | 20 | 13 | 5  |    | 38       | 11.61     |
| 2    |                   |    | 9  | 2  | 1  |    | 12       | 11.33     |
| 3    |                   | 6  | 15 | 4  | 1  |    | 26       | 11.00     |
| 4    |                   |    | 19 | 5  | 1  |    | 25       | 11.28     |
| 5    |                   | 2  | 19 | 8  |    |    | 29       | 11.21     |
| 6    |                   |    | 9  | 15 | 1  |    | 25       | 11.68     |
| 7    |                   | 6  | 8  | 3  |    |    | 17       | 11.82     |
| 8    |                   |    | 17 | 14 | 7  | 1  | 39       | 11.79     |
| 9    |                   |    | 9  | 13 | 3  |    | 25       | 11.76     |
| 10   |                   | 2  | 12 | 12 | 2  |    | 28       | 11.50     |
| 11   |                   |    | 4  | 7  |    |    | 11       | 11.64     |
| 12   |                   |    | 2  | 6  | 3  |    | 11       | 12.09     |
| 13   | 1                 | 2  | 14 | 7  | 3  |    | 27       | 11.33     |
| 14   |                   |    | 11 | 11 | 1  |    | 23       | 11.57     |
| 15   |                   | 1  | 14 | 15 | 2  |    | 32       | 11.56     |
| 16   |                   |    | 7  | 10 | 1  |    | 18       | 11.67     |
| 17   |                   |    | 2  | 9  | 14 |    | 25       | 12.48     |

**Table 2.** Frequency distribution of pelvic fin ray counts for *Notropis atherinoides* from 16 Missouri River Basin sites and Lake Erie. See Figure 1 for site location.

| Site | Pelvic fin rays |    |   |  | <i>n</i> | $\bar{x}$ |
|------|-----------------|----|---|--|----------|-----------|
|      | 7               | 8  | 9 |  |          |           |
| 1    | 1               | 35 | 2 |  | 38       | 8.03      |
| 2    |                 | 12 |   |  | 12       | 8.00      |
| 3    |                 | 26 |   |  | 26       | 8.00      |
| 4    |                 | 25 |   |  | 25       | 8.00      |
| 5    |                 | 29 |   |  | 29       | 8.00      |
| 6    |                 | 25 |   |  | 25       | 8.00      |
| 7    |                 | 17 |   |  | 17       | 8.00      |
| 8    |                 | 36 | 3 |  | 39       | 8.08      |
| 9    |                 | 23 | 2 |  | 25       | 8.09      |
| 10   | 1               | 27 |   |  | 28       | 7.96      |
| 11   |                 | 10 | 1 |  | 11       | 8.09      |
| 12   |                 | 10 | 1 |  | 11       | 8.09      |
| 13   | 1               | 24 | 2 |  | 27       | 8.04      |
| 14   |                 | 22 | 1 |  | 23       | 8.04      |
| 15   |                 | 31 | 1 |  | 32       | 8.03      |
| 16   | 1               | 17 |   |  | 18       | 7.94      |
| 17   |                 | 25 |   |  | 25       | 8.00      |

**Table 3.** Frequency distribution of dorsal fin ray counts for *Notropis atherinoides* from 16 Missouri River Basin sites and Lake Erie. See Figure 1 for site location.

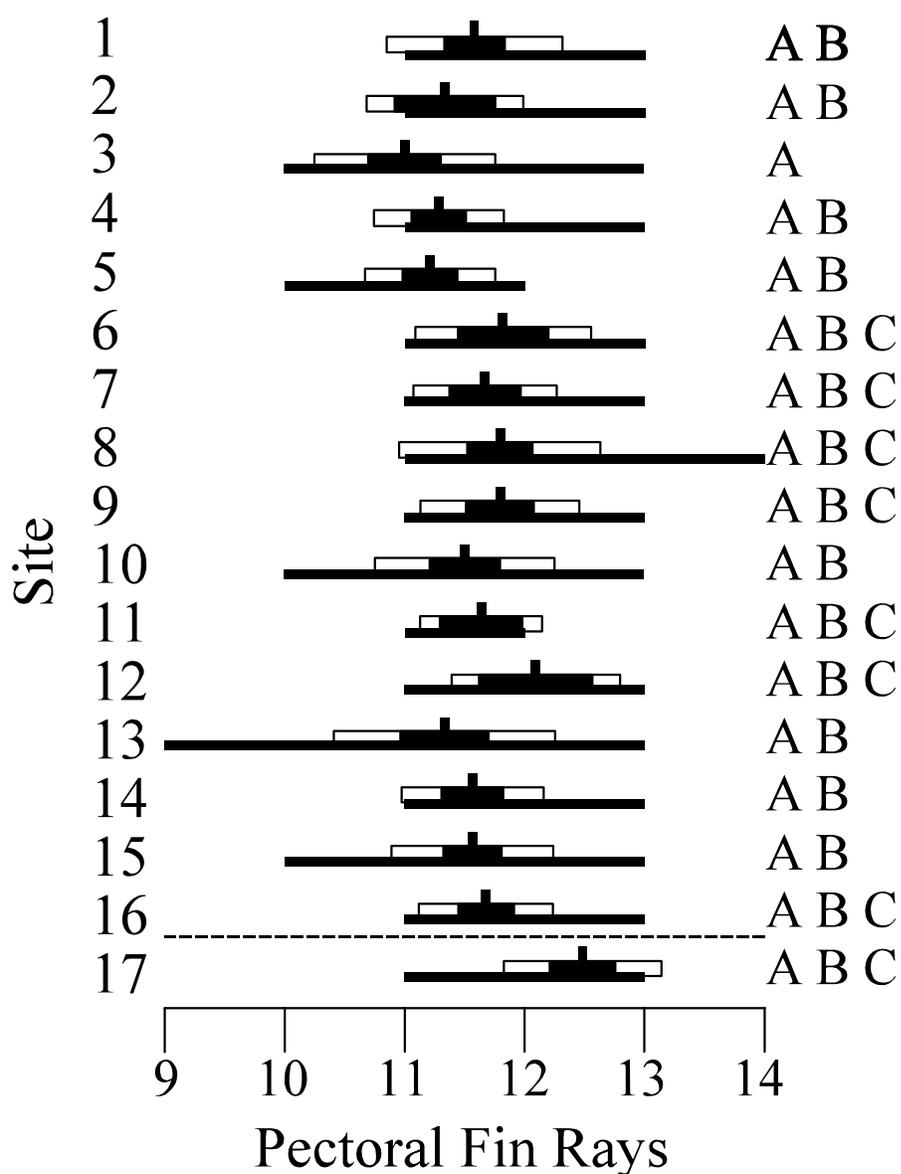
| Site | Dorsal fin rays |    |   |     | $\bar{x}$ |
|------|-----------------|----|---|-----|-----------|
|      | 7               | 8  | 9 | $n$ |           |
| 1    |                 | 38 |   | 38  | 8.00      |
| 2    |                 | 12 |   | 12  | 8.00      |
| 3    | 1               | 25 |   | 26  | 7.96      |
| 4    |                 | 25 |   | 25  | 8.00      |
| 5    |                 | 29 |   | 29  | 8.00      |
| 6    | 1               | 22 | 2 | 25  | 8.04      |
| 7    |                 | 17 |   | 17  | 8.00      |
| 8    |                 | 38 | 1 | 39  | 8.03      |
| 9    |                 | 25 |   | 25  | 8.00      |
| 10   | 1               | 27 |   | 28  | 7.96      |
| 11   |                 | 11 |   | 11  | 8.00      |
| 12   |                 | 11 |   | 11  | 8.00      |
| 13   |                 | 27 |   | 27  | 8.00      |
| 14   |                 | 23 |   | 23  | 8.00      |
| 15   | 1               | 30 | 1 | 32  | 8.00      |
| 16   |                 | 18 |   | 18  | 8.00      |
| 17   |                 | 25 |   | 25  | 8.00      |

**Table 4.** Frequency distribution of anal fin ray counts for *Notropis atherinoides* from 16 Missouri River Basin sites and Lake Erie. See Figure 1 for site location.

| Site | Anal fin rays |   |    |    |    |    | $n$ | $\bar{x}$ |
|------|---------------|---|----|----|----|----|-----|-----------|
|      | 8             | 9 | 10 | 11 | 12 | 13 |     |           |
| 1    |               |   | 9  | 26 | 3  |    | 38  | 10.84     |
| 2    |               |   | 6  | 5  | 1  |    | 12  | 10.58     |
| 3    |               | 1 | 9  | 9  | 7  |    | 26  | 10.85     |
| 4    |               |   | 11 | 13 | 1  |    | 25  | 10.60     |
| 5    |               |   | 4  | 20 | 4  | 1  | 29  | 11.07     |
| 6    |               | 1 | 7  | 14 | 3  |    | 25  | 10.76     |
| 7    |               |   | 5  | 10 | 2  |    | 17  | 10.82     |
| 8    |               | 1 | 15 | 18 | 5  |    | 39  | 10.69     |
| 9    |               |   | 8  | 13 | 4  |    | 25  | 10.84     |
| 10   |               |   | 9  | 15 | 4  |    | 28  | 10.82     |
| 11   |               |   | 3  | 7  | 1  |    | 11  | 10.82     |
| 12   |               |   | 3  | 8  |    |    | 11  | 10.73     |
| 13   | 1             |   | 3  | 17 | 6  |    | 27  | 11.00     |
| 14   |               |   | 8  | 12 | 3  |    | 23  | 10.78     |
| 15   |               | 1 | 4  | 25 | 2  |    | 32  | 10.88     |
| 16   |               |   | 4  | 12 | 2  |    | 18  | 10.89     |
| 17   |               |   | 11 | 13 | 1  |    | 25  | 10.60     |

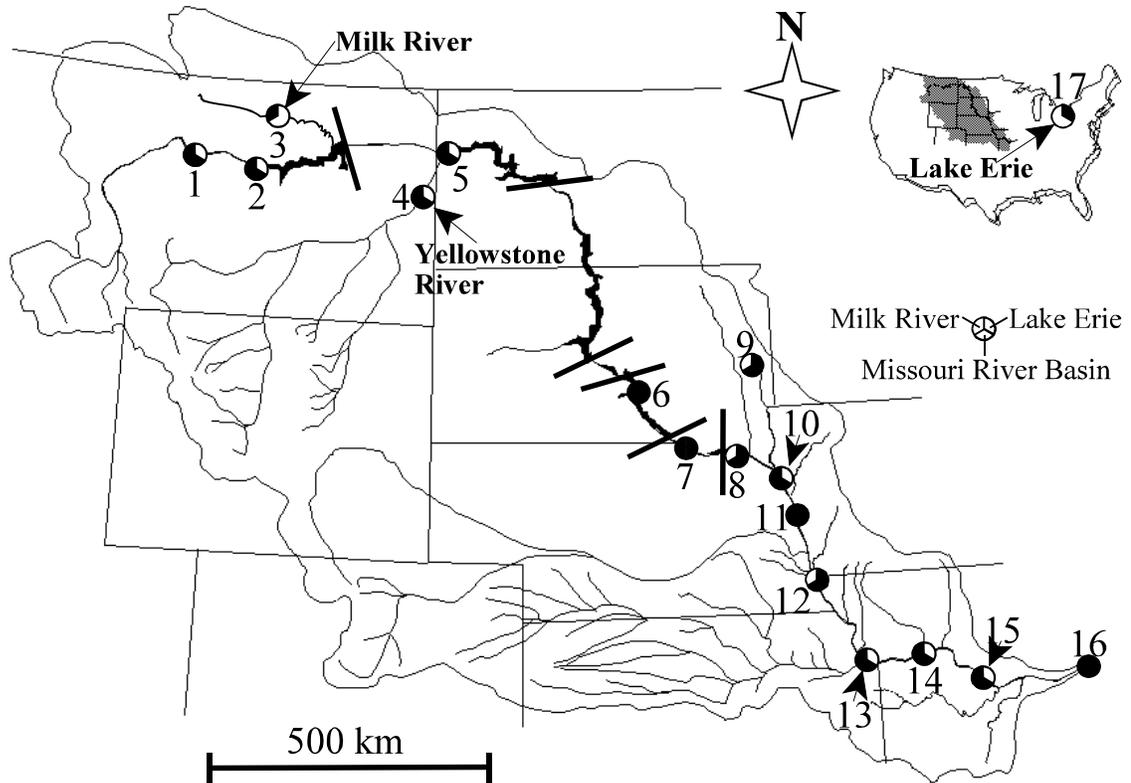
**Table 5.** Frequency distribution of vertebral counts for *Notropis atherinoides* from 16 Missouri River Basin sites and Lake Erie. See Figure 1 for site location.

| Site | Vertebrae |    |    |    |    |    |    |    |    | <i>n</i> | $\bar{x}$ |
|------|-----------|----|----|----|----|----|----|----|----|----------|-----------|
|      | 35        | 36 | 37 | 38 | 39 | 40 | 41 | 42 | 43 |          |           |
| 1    | 3         | 6  | 8  | 4  | 2  | 4  | 1  | 1  | 1  | 33       | 37.27     |
| 2    | 2         | 2  | 2  | 1  | 3  | 1  |    |    |    | 11       | 37.45     |
| 3    | 1         | 3  | 6  | 9  | 7  |    |    |    |    | 26       | 37.69     |
| 4    | 8         | 6  | 4  | 6  | 1  |    |    |    |    | 25       | 36.44     |
| 5    | 8         | 4  | 8  | 3  | 3  | 1  |    |    |    | 27       | 36.70     |
| 6    | 3         | 3  | 9  | 6  | 2  | 2  |    |    |    | 25       | 37.28     |
| 7    | 4         | 4  | 2  | 4  | 2  | 1  |    |    |    | 17       | 36.94     |
| 8    | 4         | 8  | 5  | 5  | 2  | 2  | 1  |    |    | 27       | 37.11     |
| 9    | 3         | 6  | 3  | 5  | 3  | 3  | 1  |    |    | 24       | 37.54     |
| 10   | 5         | 6  | 4  | 4  | 1  |    |    |    |    | 20       | 36.50     |
| 11   | 2         |    |    |    |    |    |    |    |    | 2        | 35.00     |
| 12   | 6         | 1  | 4  |    |    |    |    |    |    | 11       | 35.81     |
| 13   | 4         | 5  | 4  |    | 1  | 1  |    |    |    | 15       | 36.47     |
| 14   | 3         | 8  | 3  | 1  | 1  |    |    |    |    | 16       | 36.31     |
| 15   | 5         | 5  | 3  | 1  |    |    |    |    |    | 14       | 36.00     |
| 16   | 6         | 4  | 4  | 2  | 2  |    |    |    |    | 18       | 36.44     |
| 17   |           | 5  | 5  | 2  | 11 |    |    |    |    | 25       | 38.08     |



**Figure 2.** Variability in pectoral fin ray number where vertical ticks are the mean, white rectangles are  $\pm$  standard deviation, black rectangles are  $\pm$  standard error, and horizontal lines are the range. Site number locations are mapped in Figure 1. Sites with an “A” to the right are significantly different from site 17 (Lake Erie). Sites with a “B” are not significantly different from each other. Sites with a “C” to the right are significantly different from site 3 (Milk River).

All five of the Upper Missouri River and four of the seven Lower Missouri River sites had significantly lower mean pectoral fin ray counts than the Lake Erie site (17). No mainstem Missouri River sites significantly differed from one another (Figure 3).



**Figure 3.** Geographic differences in pectoral fin ray number. Circles on map indicate sample sites. A white third represents a significant difference while a black third represents no significant difference between the site and the designated third. The six lines show the location of the mainstem dams on the Missouri River.

Vertebrae number ranged from 35 to 43 among all sites (Table 5). Site 11 was excluded from the analyses because only two individuals were counted from that site. Like pectoral fin rays, vertebrae of emerald shiners from Lake Erie (site 17) and the Milk River (site 3) showed significant differences among sites ( $P < 0.001$ ). Vertebrae counts for emerald shiners from site 1 in the Upper Missouri River also differed from other sites ( $P < 0.001$ ). Lake Erie (site 17) emerald shiners had the highest number of mean vertebrae and differed from emerald shiners of the Yellowstone River (site 4) and five of the six Lower Missouri River sites (10, 12, 14, 15, and 16). The Milk River (site 3) emerald shiners only differed from site 12 while site 1 in Montana differed from both sites 12 and 15 in the Lower Missouri River. Site 12 emerald shiners were unique in that they had the highest mean pectoral fin ray count of any Missouri River Basin site, yet they had the overall lowest mean vertebrae number.

Latitude, water temperature, and turbidity were the only habitat variables that showed a significant correlation with pectoral fin ray number. Latitude and degree days showed a strong negative linear correlation ( $P < 0.0001$ ;  $r^2 = 0.98$ ). As expected, the number of degree days increased from northern to southern latitudes (Figure 4A). As latitude increased, pectoral fin ray number followed a parabolic path, peaking in the middle latitudes and then decreasing toward the more northern and southern latitudes (Figure 4B). I fit the data with non-linear polynomial regression using a quadratic model ( $y = 1.718x - 0.020x^2 - 24.535$ ) to reveal the parabolic correlation ( $P = 0.006$ ;  $r^2 = 0.52$ ). Degree days had a strong linear correlation with latitude so pectoral fin rays also followed a parabolic path across sites as a function of degree days (Figure 4C). This correlation

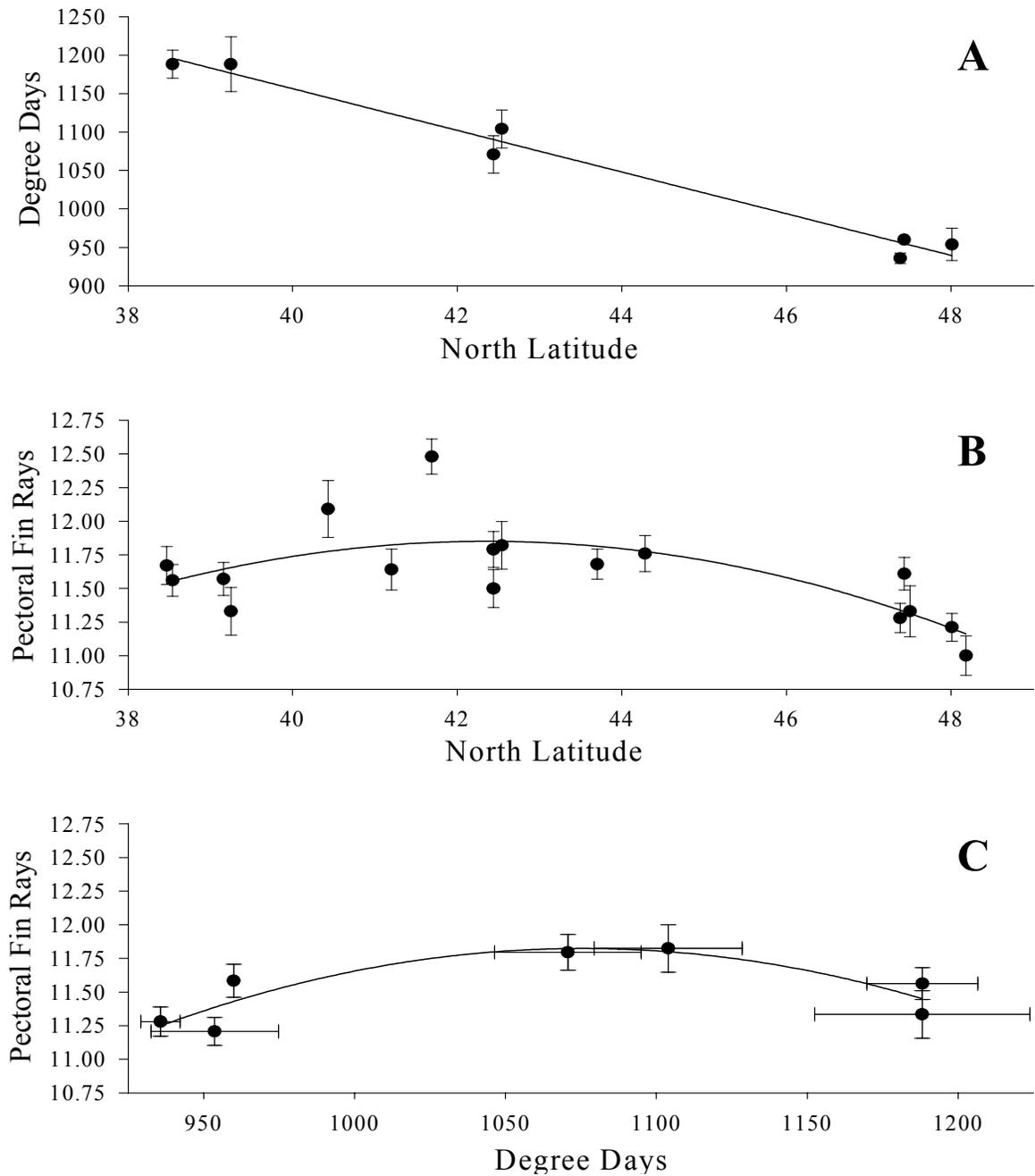


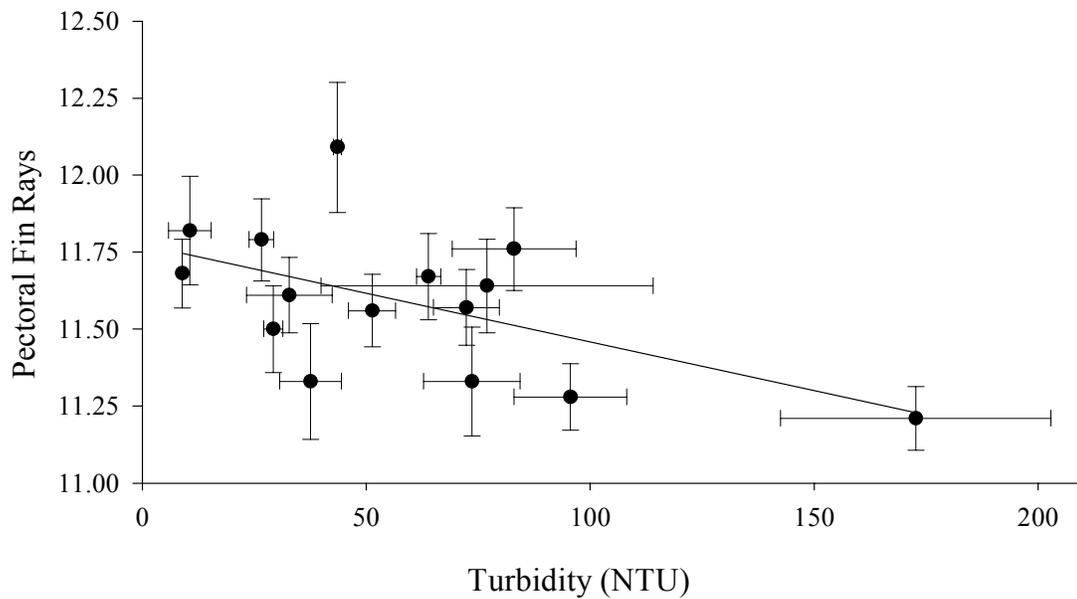
Figure 4. Relationships between latitude, degree days, and pectoral fin rays across sites. Vertical and horizontal bars represent standard error.

was not significant at  $\alpha=0.05$  ( $P=0.054$ ;  $r^2=0.77$ ) because fewer data were available, but it clearly showed the same pattern ( $y=0.0634x - 2.945E10^{-5}x^2 - 22.249$ ). Both the middle latitudes and the middle range of degree days corresponded to higher mean pectoral fin ray numbers. Although the latitude and degree day data showed the same pattern, they were not collinear.

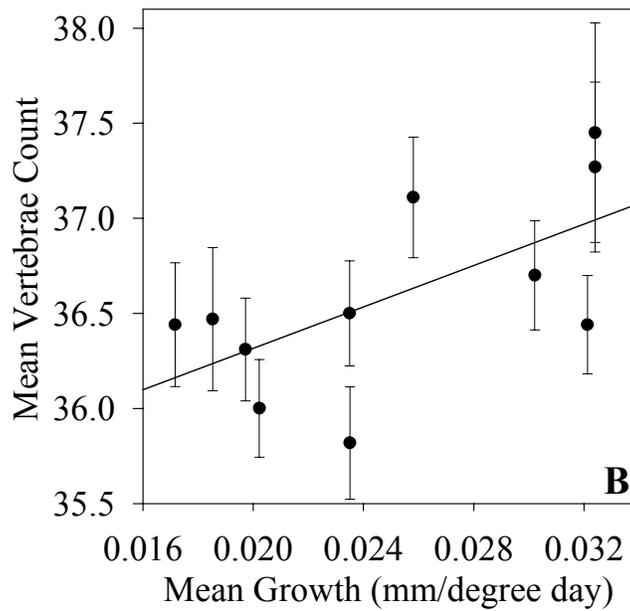
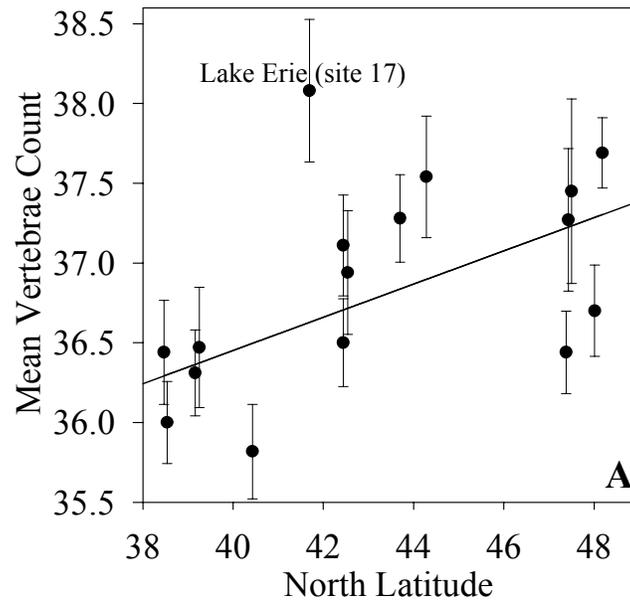
As turbidity increased across sites, pectoral ray number decreased (Figure 5). In contrast to latitude and degree day correlations, pectoral rays shared a negative linear correlation with turbidity ( $p=0.039$ ;  $r^2=0.309$ ). However, if the apparent outlying data point with the highest NTU value is dropped, the relationship loses significance ( $p=0.039$ ;  $r^2=0.309$ ). The lowest turbidities were in the Middle Missouri River and the greatest turbidities were in the Lower Missouri River. The inverse of turbidity is transmittance, which is a measure of light penetration through water. Therefore, the turbidity relationship suggests that pectoral ray number may increase with underwater light intensity. All other habitat variables I measured were analyzed in multiple linear and polynomial regression models, but no combinations were significant.

Vertebrae showed correlations with both latitude and growth rate. Lake Erie (site 17) was not included in these regressions, but was plotted on figure 6A. Vertebrae had a positive linear relationship with latitude and increased from southern to northern latitudes ( $p<0.001$ ;  $r^2=0.44$ ). The regression showed a predicted mean increase of approximately one vertebrae per ten degrees of latitude (Figure 5A). Growth rate was also linearly correlated with mean vertebrae number ( $p=0.037$ ,  $r^2=0.40$ ). Higher daily growth rates, standardized by degree days, were associated with higher mean vertebrae number (Figure

6B). Multiple regression with both latitude and growth was not significant, yet it revealed a variance inflation factor of 18.235, indicating the two variables were close to being collinear. These two linear relationships of vertebrae with latitude and growth differed from the two parabolic curves of pectoral fin rays with latitude and degree days and showed the independent variability between the two meristic characters.



**Figure 5.** Pectoral fin rays a function of mean turbidity across sites for *Notropis atherinoides*. Vertical and horizontal bars represent standard error.



**Figure 6.** Relationships of mean vertebrae number for *Notropis atherinoides* to degrees north latitude and mean growth measured as mm grown per degree day. Bars represent standard error.

## DISCUSSION

The variability of pectoral fin rays and their correlation to multiple habitat variables made it clear that there is not a single deterministic force that can predict the direction of meristic change. The pectoral (paired) and anal (medial) fin rays had high variability, while the pelvic (paired) and dorsal (medial) fin rays had low variability suggesting that body placement and usage have no discernable relationship with meristic variation. Lindsey (1954) and Smith and Bailey (1961) also showed that meristic features on an individual can vary independently from one another.

The implications of an inverse relationship between turbidity and pectoral fin ray number are questionable. However, I can not rule out the possibility that the relationship may reflect any number of single or multiple correlations to other variables upon which turbidity is dependent. At the levels I measured, turbidity most affects fish by limiting the amount of light penetration into the water and limiting visibility. Light intensity during the embryonic stage has been shown to vary inversely with meristic numbers (McHugh 1954b; Eisler 1961). My data and inferences about light intensity would contradict those findings leading me to conclude that the relationship is coincidental and more likely correlated to one or more of variables that affect turbidity.

The meristic values for fin rays of Lake Erie fish concur with those of Flittner (1964) who sampled the same area. All Missouri River fin ray counts were consistent with fish from Canadian lakes except for pectoral fin rays (Scott and Crossman 1973). Missouri River values were much lower than their reported range of 13 to 17 and may constitute recognition of a new lower range for pectoral fin ray number. Of course, when

considering a much larger geographic range, perhaps the lower pectoral fin ray numbers in the entire Missouri River Basin were a true natural cline and were lower than those of their Canadian conspecifics.

Water temperature is often thought to be the most influential factor in the environmental control of meristic numbers. Previous studies on pectoral fin ray numbers have found relationships with water temperature and latitude to be a positive linear correlation (McHugh 1954a; Quast 1964), a negative linear correlation (Lindsey 1954), or in a laboratory setting, a peaking parabola (Tåning 1944, 1952). Other meristic characters show a wide range of responses (Fowler 1970). Although I do not have precise temperature data, the relationship with degree day data would support the laboratory findings of Tåning. However, the results were likely affected by the type of temperature data used. The gauging stations recording temperatures were located along main channel shorelines at different depths. These locations did not necessarily reflect the water temperature at actual spawning sites. Emerald shiners spawn in a variety of habitats, yet in the middle river segment I consistently found schools of emerald shiners, 6-15 mm in length, in water less than 0.5 m deep along and between sandbars where flow was negligible. That length range indicates that those fish were likely between 4 and 12 days old (Flittner 1964) and incapable of swimming to the sandbar against the river current. Therefore, I assume that the shallow sandbar habitat was being used for spawning and early rearing. Shallow sandbar habitat was less available in the Upper Missouri River where the channel is more confined, and is nearly absent from the channelized Lower Missouri River. I have measured 5-9°C temperature differences between main channel

and sandbar habitats on warm, sunny days. I think it is likely that the majority of emerald shiners from the Middle Missouri River are using this warmer sandbar habitat for spawning. If I had true temperature data at the exact time and site of spawning and development, I believe that it would show a more positive linear relationship with pectoral fin rays than what I found. A positive relationship would suggest pectoral fin ray numbers increase when embryos develop in warmer habitat and support the findings of McHugh (1954a) and Quast (1964).

The results of the latitude correlation with pectoral fin ray number were paradoxical. Many studies have shown the positive clinal relationship between latitude and meristic counts and assumed it to be a result of gradients in temperature and day length. However, I found a parabolic relationship indicating a peak in pectoral fin ray number across middle river sites while decreasing toward both upper and lower river sites. This disruption in the natural cline suggests that some type of phenomenon is different in the Middle Missouri River segment than in the upper and lower segments. The degree day data show that main channel water temperatures increased gradually from upper to lower river so that the only way the data could relate to temperature would be if the warm sandbar habitat explanation was true.

The availability of specific habitats as an explanation for ray differences seems to be supported by turbidity data too. I suspect that turbidity does not have a direct effect on pectoral fin ray data, but that it may reflect other environmental conditions contributing to the meristic variation. The data showed that the lower turbidities corresponded to higher pectoral fin ray counts. Turbidity is a function of river gradient, depth, velocity, and

substrate. Of those, I believe velocity best distinguishes the Upper, Middle, and Lower Missouri River. Velocity is lowest in the Middle Missouri River because of impoundment and a less confined channel. Velocity is higher in the upper and lower river because it is naturally more confined by bluffs in the Upper Missouri River and is highly confined by dikes and levees in the Lower Missouri River. These basin attributes cause increased channel width, higher sinuosity, and lower velocity in the middle river resulting in the production of the unique sandbar habitats. Knowing that emerald shiner fry were found in sandbar habitats of the Middle Missouri River, I believe that they may be forced to spawn and rear under less than favorable conditions in other parts of the river. Physical habitat and its associated local conditions appear to be the most likely explanation for the differences I observed in pectoral fin ray number.

The vertebrae data followed a pattern often seen in other field studies (Fowler 1970). The clinal increase with latitude was linear, yet the mid-latitude sites in the Middle Missouri River all had counts that produced positive regression residuals. Although there was not a statistically significant parabolic relationship between latitude and vertebrae number as seen with the pectoral fin data, the mid-latitude rise was worth noting. The Lake Erie site had the greatest number of vertebrae ranging from 36 to 39. This range overlapped the data of Flittner (1964) who found a range of 38 to 42 in emerald shiners from western Lake Erie. Bailey and Allum (1962) summarized vertebrae counts of emerald shiners from throughout their United States range from 1896 to 1945. Sites 2 or 5 would be the closest sites to compare to their data although my samples were not only temporally, but physically separated by the presence of a dam in Montana.

Nonetheless, vertebrae counts ranged from 35 to 40 for those sites compared to the five samples of Bailey and Allum having either 39 or 40 vertebrae. These consistencies among studies suggest that no substantial temporal changes have occurred and that my data reflect accurate measurements across all sites. The linear cline I found therefore indicates that vertebrae numbers have likely not been affected by river alteration. Any changes that may have occurred, such as my speculation about the mid-latitude rise in vertebrae number, were not significant changes from either historic or natural vertebral clines.

The association between vertebrae and growth was likely covariant with latitude. The high variance inflation between latitude and growth suggests that growth was a function of latitude and thus simply a redundant measure. Growth like latitude encompasses many environmental variables, any number of which could have been responsible for the variation seen among sites. However, the prevailing thought that temperature is the functional part of latitudinal differences is contradicted here. Growth rate increased with latitude even after being standardized for temperature. This may suggest that conditions that favored growth in an area of the river may also have favored vertebrae development. Typically, faster growth rates result in fewer meristic features (Gabriel 1944). However, a relationship between growth and vertebral number may coincide with the pleomerism phenomenon. In two populations where one is found to have a larger mean body size than the other, meristic counts tend to be higher among the larger bodied population (Lindsey 1975). Growth differences would support larger sized northern fish having a higher mean vertebrae number than the fish from southern sites.

This is also supported by the shorter mean lengths of emerald shiners from the lower latitude sites. Although temperature standardized growth rate was linearly related to vertebrae number, it does not necessarily explain the variation among sites. However, it does imply that temperature was not the primary deterministic force in vertebral variation of Missouri River emerald shiners.

Although the data did not statistically identify groups of emerald shiners from any portion of the mainstem Missouri River as characteristically distinct from one another, the correlated pattern of means within that variation suggests that the variation was not random. Vertebral clines were easily explained as either the result of latitudinal changes in habitat or pleometric increases related to growth. Both cases are natural and seem to be uninterrupted by habitat alteration. There are two scenarios that can explain the pectoral fin ray results. First, the geographic size of the Missouri River Basin may not be large enough to reveal a distinct cline in this meristic attribute and would naturally show no appreciable or patterned differences. Second, the Lower Missouri River that formerly had the same types of habitat as the Middle Missouri River may have historically had similar meristic values. In either case, river alteration seems to have disrupted a clinal absence or increase with latitude and created the peaking parabolic relationship. The anomalous Middle Missouri River segment corresponds to the serial discontinuity concept and shows how disruption in natural habitat gradients can also affect a natural meristic gradient in a population. These small differences in pectoral fin ray number between sites probably do not provide an advantage or cause a hindrance to the local fish, but they are important in gauging the effects of habitat on development. The differences

reflect how alteration and fragmentation of an historically continuous habitat may have caused localized responses in a species.

Meristic studies were originally used for population identification and systematics, but have been almost abandoned in light of the advances in genetics over the past two decades. In this study, I have been able to use meristic counts not as a population or systematics tool, but instead as a possible indicator of phenotypic responses to habitat alteration. Meristic counts are a function of growth rates in embryos and larvae, so the data should be considered by managers as a reflection of how river regulation may affect the growth and development during the early life history of fishes.

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## Chapter 4

Morphometric Variation among Emerald Shiners (*Notropis atherinoides*)  
in the Central United States

## ABSTRACT

Emerald shiners (*Notropis atherinoides*) from the Missouri River and Great Lakes basins were compared to determine whether morphometric differences distinguished the populations. The habitats at different sites ranged from channelized riverine to lacustrine. Emerald shiners were once thought to comprise a lake and river sub-species based on differences in morphology. I used fish from habitat extremes to test the hypothesis that differences in habitat among sites were capable of producing alternate morphotypes adapted to local conditions. The sheared box-truss method was used for size-free discrimination among morphotypes. I also made eye, head, and jaw measurements. Box-truss results revealed little difference in shape among sites. Individual shape variation was higher in the Missouri River than in lake, reservoir, and smaller tributaries allowing for only marginal discrimination among three sites. Eye, head, and jaw measurements differed independently from one another thereby producing many morphotypes. Larger

eye sizes were associated with the least turbid sites and reservoir sites where diets shifted to zooplankton rather than periphyton. The higher individual shape variation among the Missouri River emerald shiners may reflect their adaptability to variable habitat conditions. Except for the eye size of Lake Francis Case fish, alteration of the Missouri River has not substantially affected or caused divergence in the morphology of emerald shiners.

## INTRODUCTION

The morphology of an organism is the product of genetic architecture and the environment (Strauss 1987; Scheiner and Lyman 1989; Motta and Kotrschal 1992). The environment provides selective pressures that favor certain phenotypes while inheritance constrains the range of phenotypic expression that organisms possess. This range of phenotypic expression or “norm of reaction” may be different among traits, individuals, or populations (Schmalhausen 1949). If a norm of reaction is great enough in the presence of environmental conditions that favor or isolate opposite phenotypic extremes, then phenotypic differentiation will result from one of three processes: 1) natural selection may favor phenotypic variants at one or both ends of the norm of reaction, 2) genetic drift may result in random phenotypic divergence regardless of selective pressures, or 3) plasticity may allow individuals to shift within their phenotypic norm of reaction without requiring or initiating concomitant genetic changes. Natural selection results from physical, chemical, and biological conditions producing stresses capable of preventing ill-adapted individuals from reproducing (Darwin 1898). Genetic drift

requires no or negligible gene flow between populations insuring that unique alleles are retained by and only expressed in specific populations (Wright 1931). Plasticity, unlike the other two processes, can happen within a generation when individuals shift their phenotypic expression within their norm of reaction as an adaptive response to local conditions (Stearns 1989). The inherent norm of reaction provides a phenotypic range in which individuals randomly vary around a population mean, but that mean is subject to change within the norm of reaction when sufficient stimuli initiate a phenotypic shift.

I selected the Missouri River, a highly fragmented yet continuous habitat, to determine if individuals in different habitats have responded phenotypically to environmental alteration. The historic Missouri River flowed uninterrupted for 3500 km from north central Montana to the mouth near St. Louis, Missouri. The historic river gradually changed from a mountain stream, to a confined prairie river in Montana and the Dakotas, to a wide meandering floodplain river through Nebraska and Missouri. Between 1937 and 1963, the Missouri River was transformed by six mainstem dams resulting in the impoundment of 1095 km of the river and by channelization of the lower 1212 km of the river (Hesse et al. 1989; Schmulbach et al. 1992). These alterations have produced a system of lakes and higher velocity river channels while leaving a portion relatively unimpacted.

Past studies have found that diverse lotic habitats can produce divergent intraspecific morphotypes (Williams and Wilde 1981; Balon 1992; Kelsch 1995; Wood and Bain 1995; Golubtsov et al. 1999). Each of these studies attributed intraspecific morphological differences to habitat differences. Balon (1992) even proposed that

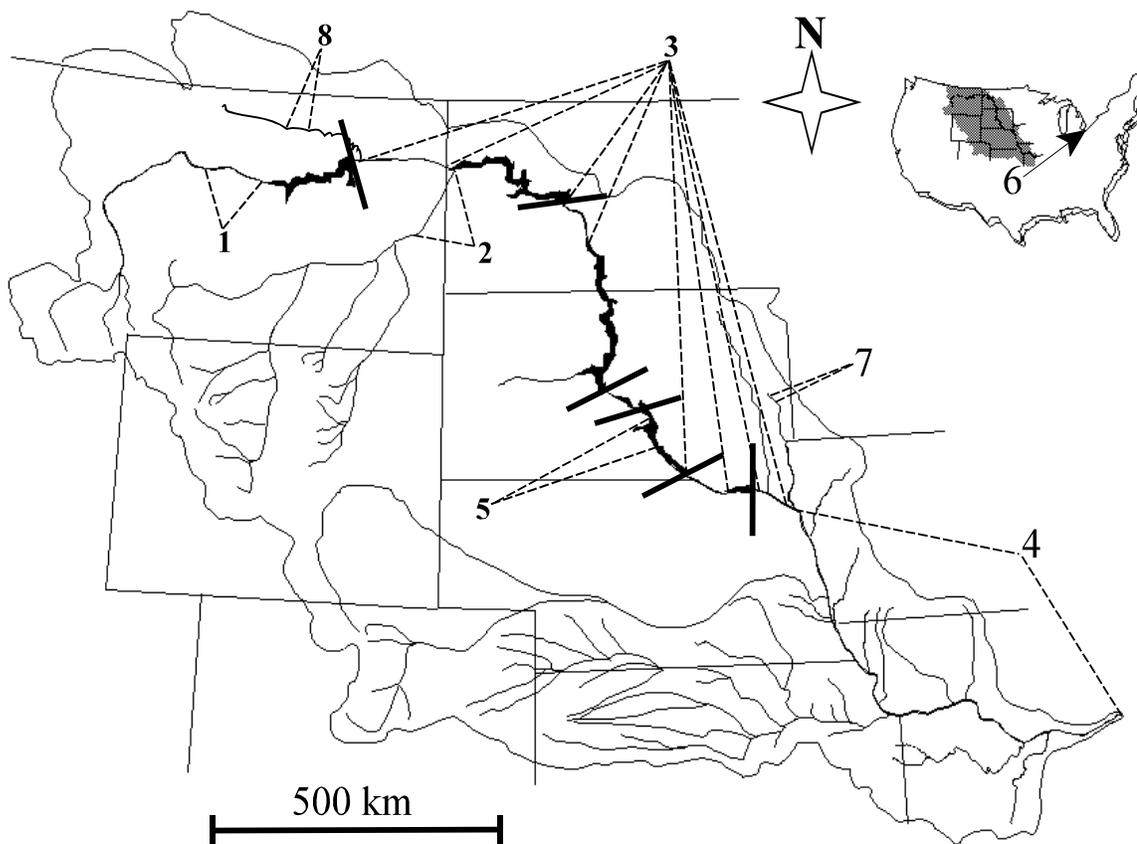
damming of the River Danube in Hungary may have produced sub-species of *Gobio albipinnatus*, each conforming morphologically to fit their new habitat. These studies also show that complete isolation is not required for divergent adaptation to occur. Considering these findings, the diverse and fragmented habitat conditions in the Missouri River seem conducive to the production of alternate morphotypes.

Emerald shiners historically inhabited the entire Missouri River and remain in all Missouri River habitat types (Bailey and Allum 1962; Pflieger and Grace 1987; Young et al. 1997). Previous surveys and research have found a wide range of morphotypes throughout the Mississippi River, Great Lakes, Hudson Bay, and Mackenzie River basins that compose the range of this species (Bailey and Allum 1962; Flittner 1964; Scott and Crossman 1973). Hubbs (1945) proposed the existence of a very similar species, *Alburnellus percobromus*, ranging throughout the plains states. However, this delineation was refuted as simply the intraspecific variation of *Notropis atherinoides* (Bailey and Allum 1962). Later, Hubbs and Lagler (1964) proposed sub-specific classification within *Notropis atherinoides* based on differences in morphotypes between Great Lakes and Mississippi River basin populations, but this was also refuted as being only variable forms of a highly morphologically plastic species (Flittner 1964). Sexual dimorphism is often a problem in morphology studies, but sexual differences in emerald shiners are present only during spawning periods (Flittner 1964). This apparent propensity for phenotypic variation and lack of sexual dimorphic features favors the use of emerald shiners. They have a sufficient norm of reaction in which I can measure potential adaptive shifts in mean phenotype. If emerald shiners do have a relatively high degree of

morphometric plasticity, then the fragmented and transformed habitats of the Missouri River may incite phenotypic shifts and produce distinctive morphotypes among the different habitat types.

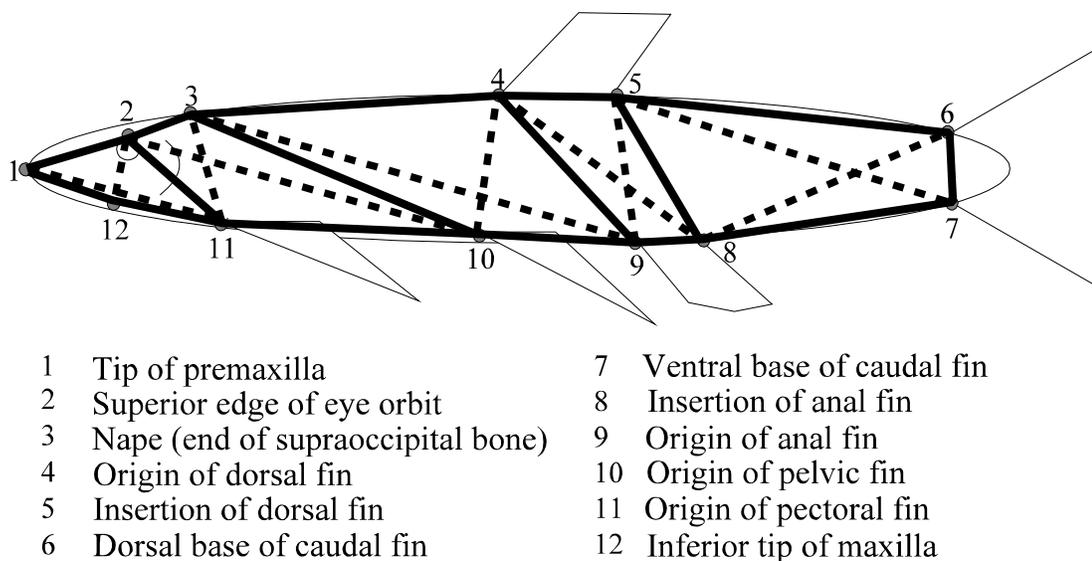
## METHODS

Emerald shiners were collected from the Missouri River, the Yellowstone River, the Milk River (Montana), the Big Sioux River (South Dakota), and Lake Erie (Figure 1). The Missouri River was divided into three regions based on hydrology and habitat: the upper unimpacted Missouri River (site 1), the middle inter-reservoir Missouri River (site 3), and the lower channelized Missouri River (site 4). The decision to pool samples in sites 3 and 4, each covering wide ranges and a 3 year period, was verified by checking for between year or sub-site differences in standard length and weight using  $K_n$  for simple condition factor (Le Cren 1951). The remaining sites did not share physiographic or hydrologic similarities and were not considered for pooling. All emerald shiners were collected between July and October of 1996-1998 in the Missouri River Basin and in March 1998 for the Lake Erie individuals. Emerald shiners were collected with 9.53-mm bar mesh, 30-m bag seines and by electrofishing. Only fish with a total length greater than 30 mm and representing various size classes were used for analysis. Samples were fixed in 4% formalin and preserved in 70% ethanol.



**Figure 1.** Site locations in the Missouri River Basin and Lake Erie. The six lines crossing the Missouri River show the location of mainstem dams. Emerald shiners were collected from various points within the range(s) indicated by the dotted lines. Site 1 is the Missouri River from Arrow Creek to Beauchamp Coulee in Montana. Site 2 is the Yellowstone River from the diversion dam to the mouth. Site 3 includes four separate inter-reservoir sections on the Missouri River: Milk River to Yellowstone River, Garrison Dam to Lake Oahe, Fort Randall Dam to Lewis and Clark Lake, and Gavins Point Dam to Ponca, Nebraska. Site 4 is the channelized portion of the Missouri River and stretches from Ponca, Nebraska to the mouth. Site 5 is Lake Francis Case. Site 6 is Lake Erie at the mouth of the Maume River in Toledo, Ohio. Site 7 is the Big Sioux River in Brookings County, South Dakota. Site 8 is the Milk River near Glasgow, Montana.

A box truss protocol was used to identify differences in overall body shape among sites (Bookstein et al. 1985). Landmarks were chosen at fixed points (Figure 2). Each fish was secured on a card and a pin inserted to leave a perforation at each of the twelve landmarks. The perforated cards were then digitized to obtain Cartesian coordinates for each of the landmarks from which all elements of the box truss network could then be calculated (each point-to-point distance is termed an element).



**Figure 2.** Location of the 12 fixed landmarks that form the five quadrilaterals comprising the box-truss set of measurements.

The 26 individual elements were  $\log_{10}$  transformed and analyzed using a size-sheared principal components analysis (PCA) method to identify shape differences (Strauss and Bookstein 1982; Bookstein et al. 1985; Humphries et al. 1981). The shearing method assumes that the first component will represent size or length of the fish. A regression of PC1 (size) on mean-centered principal components provides coefficients used to recalculate scores of the remaining components, free from size influence. The second and all remaining recalculated component scores were then plotted against standard length to verify size-free shape discrimination. Size-free components were plotted against each other to identify any groupings or discrimination among sites. The eigenvector of each truss element identified which elements contributed to the sheared components that provided discrimination. If plots showed apparent discrimination, then a multivariate analysis of variance (MANOVA) was used to detect statistical discrimination, and discriminant function analysis (DFA) was used to determine proportional overlapping between sites.

Three additional morphometric measurements were made that could not be covered in the box truss network. Head depth at pupil, eye diameter, and jaw width were measured with digital calipers. Each measurement was standardized for size differences using the equation:

$$D_t = \log_{10} D - \beta(\log_{10} SL - \log_{10} SL_m),$$

where  $D_t$  is the transformed measurement,  $D$  is the original measurement,  $\beta$  is the slope of the regression of  $\log_{10} D$  on  $\log_{10} SL$ ,  $SL$  is standard length, and  $SL_m$  is the overall mean

of standard length (Reist and Crossman 1987). The transformed head, eye, and jaw variables were individually tested for differences among all pairs of sites using an analysis of variance (ANOVA). A MANOVA then determined site-pair differences for the composite of all three measurements. Lastly, a DFA with cross-validation classification was used to find how much potential 3-dimensional overlap existed between site pairs as measured by proportional incorrect classification of data.

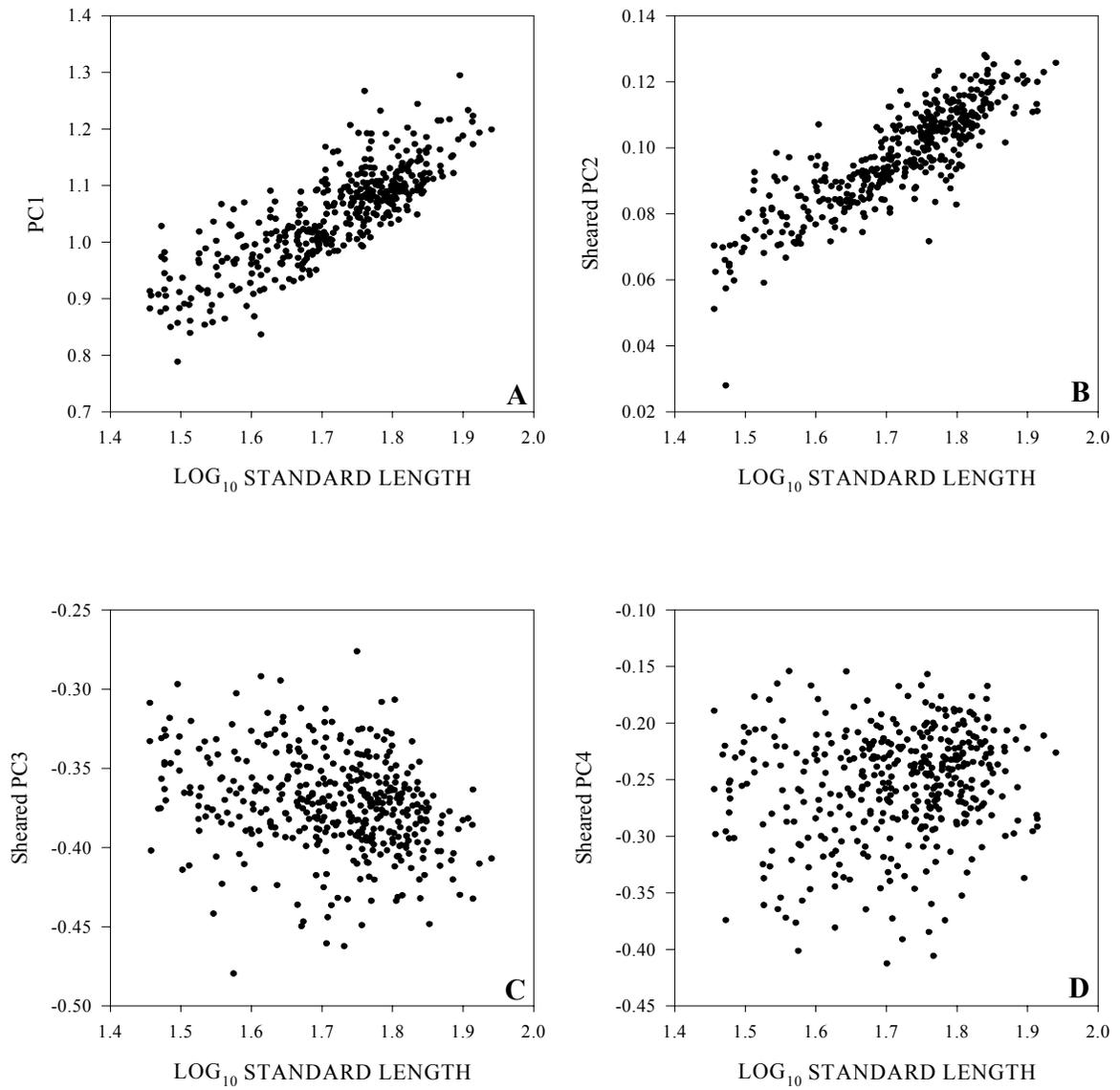
## RESULTS

I used a total of 410 emerald shiners for analysis. Among the eight sites, total lengths ranged from 36.7 to 103.7 mm. Mean length per site differences were significant ( $p < 0.0001$ ) and variance differences among sites exceeded  $3\sigma$  in four instances. There was complete overlap in total length among all sites for a range of less than 10 mm.

The first PCA on  $\log_{10}$  transformed truss elements loaded high for all eigenvectors on PC1 showing that variation in all truss elements were being explained by PC1 (Table 1). As expected, the PC1 scores were directly correlated to standard length (Figure 3A). The shearing procedure then produced new component scores for PC2, PC3, and PC4. Sheared PC2 remained heavily influenced by size and closely matched PC1 (Figure 3B). The eigenvectors for sheared PC2 continued to load evenly across most truss elements indicating variation in most measurements were again explained by PC2 (Table 1). Sheared PC3 and sheared PC4 were effectively separated from the effects of size (Figure 3C and 3D). These were the only two principal components that had eigenvectors that loaded high on less than four truss elements thus identifying the variation of those

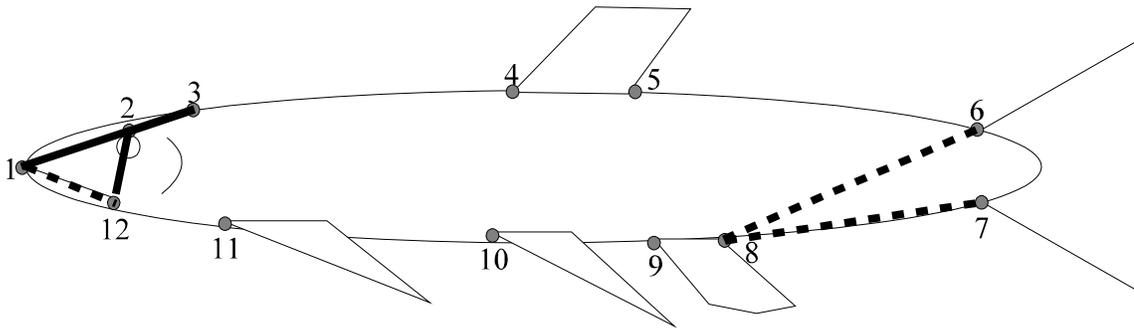
**Table 1.** Eigenvector loadings for each of the truss elements on the original and sheared principal components (PC) and eigenvalues showing the percent of the total variance each component explains. Element numbers refer to landmarks on body (Figure 2).

| Element                               | PC1   | PC2    | PC3    | PC4    | Sheared PC2 | Sheared PC3 | Sheared PC4 |
|---------------------------------------|-------|--------|--------|--------|-------------|-------------|-------------|
| 1-2                                   | 0.170 | -0.114 | -0.290 | 0.047  | -0.039      | -0.263      | 0.123       |
| 1-11                                  | 0.160 | -0.022 | 0.026  | 0.060  | -0.026      | 0.055       | 0.124       |
| 1-12                                  | 0.200 | -0.838 | 0.150  | 0.352  | -0.130      | 0.188       | 0.306       |
| 2-3                                   | 0.143 | 0.131  | 0.510  | 0.030  | -0.006      | 0.542       | 0.098       |
| 2-10                                  | 0.193 | 0.031  | 0.047  | -0.038 | -0.025      | 0.082       | 0.090       |
| 2-11                                  | 0.159 | 0.003  | 0.050  | 0.058  | -0.023      | 0.079       | 0.122       |
| 2-12                                  | 0.177 | -0.004 | -0.775 | 0.154  | -0.027      | -0.752      | 0.185       |
| 3-4                                   | 0.216 | 0.020  | 0.028  | -0.028 | -0.030      | 0.067       | 0.108       |
| 3-9                                   | 0.216 | 0.014  | -0.001 | -0.054 | -0.030      | 0.038       | 0.094       |
| 3-10                                  | 0.208 | 0.045  | 0.005  | -0.022 | -0.026      | 0.042       | 0.107       |
| 3-11                                  | 0.186 | 0.089  | 0.063  | 0.136  | -0.017      | 0.097       | 0.180       |
| 4-5                                   | 0.173 | 0.028  | -0.060 | -0.166 | -0.022      | -0.030      | 0.008       |
| 4-8                                   | 0.212 | -0.005 | -0.022 | -0.221 | -0.032      | 0.016       | 0.001       |
| 4-9                                   | 0.217 | 0.020  | -0.006 | -0.063 | -0.030      | 0.033       | 0.090       |
| 4-10                                  | 0.219 | 0.028  | 0.044  | 0.028  | -0.029      | 0.084       | 0.141       |
| 5-6                                   | 0.194 | 0.144  | 0.047  | 0.092  | -0.011      | 0.083       | 0.161       |
| 5-7                                   | 0.200 | 0.108  | 0.014  | 0.066  | -0.017      | 0.050       | 0.150       |
| 5-8                                   | 0.232 | -0.010 | 0.006  | -0.206 | -0.036      | 0.048       | 0.021       |
| 5-9                                   | 0.219 | 0.038  | 0.032  | -0.039 | -0.028      | 0.071       | 0.104       |
| 6-7                                   | 0.233 | -0.182 | 0.067  | -0.086 | -0.056      | 0.110       | 0.087       |
| 6-8                                   | 0.172 | 0.211  | 0.079  | 0.361  | -0.000      | 0.111       | 0.295       |
| 7-8                                   | 0.155 | 0.299  | 0.032  | 0.516  | 0.013       | 0.060       | 0.370       |
| 8-9                                   | 0.199 | -0.049 | 0.007  | -0.512 | -0.035      | 0.043       | -0.165      |
| 9-10                                  | 0.231 | -0.042 | -0.010 | -0.080 | -0.039      | 0.031       | 0.088       |
| 10-11                                 | 0.217 | 0.048  | 0.011  | -0.088 | -0.026      | 0.050       | 0.076       |
| 11-12                                 | 0.151 | 0.215  | -0.039 | 0.009  | 0.003       | -0.012      | 0.091       |
| Eigenvalue                            | 0.225 | 0.012  | 0.008  | 0.006  | -           | -           | -           |
| Proportional<br>Variance<br>Explained | 0.833 | 0.039  | 0.003  | 0.002  | 0.833       | 0.039       | 0.003       |



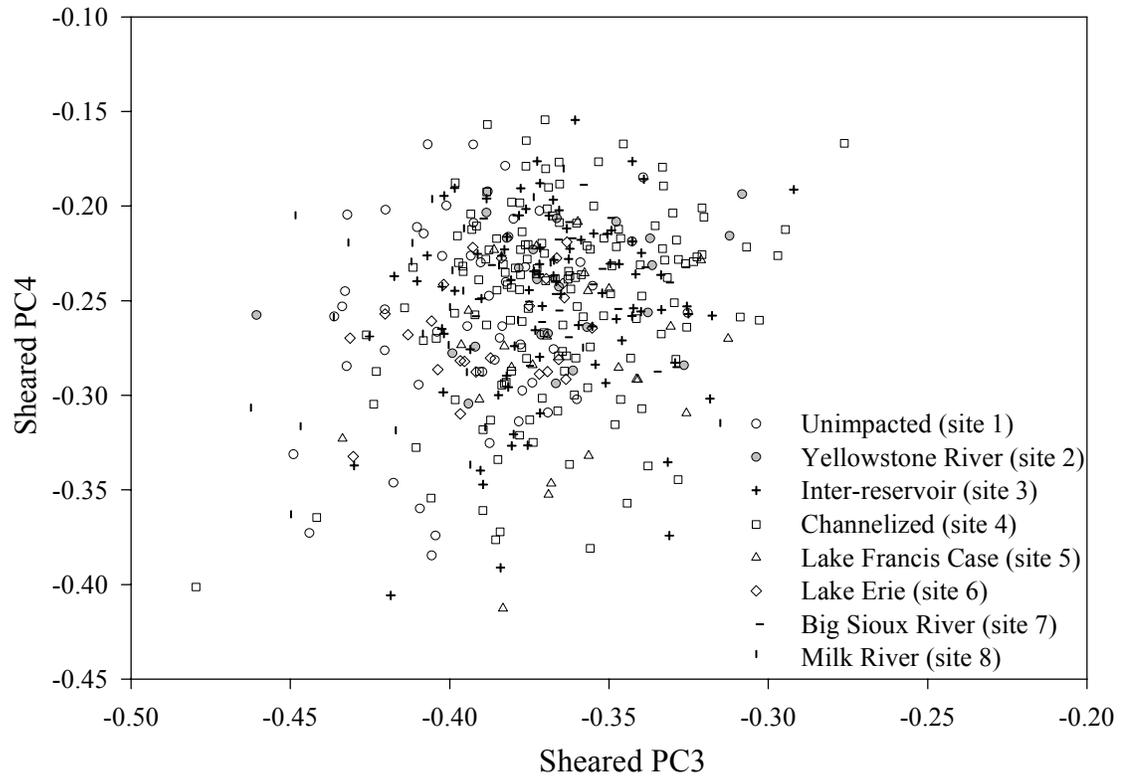
**Figure 3.** Relationship between standard length and principal components. Data from all sites appear in each graph.

elements as being specifically explained by their associated component (Table 1). For Sheared PC3, elements 2-12 and 1-2 decrease while element 2-3 increases. As a result, these three truss elements form a composite that represents movement of the eye in a forward and downward placement on the head (Figure 4). For sheared PC4, elements 7-8, 6-8, and 1-12 all increase and represent an elongation of the posterior half of the body and an elongation in either snout length or jaw length (Figure 4). The total variation explained by sheared PC3 was 0.028 and 0.018 for sheared PC4, while size variation explained by PC1 and PC2 accounted for 0.872 of the variation. Sheared principal components 3 and 4 explained less than five percent of the total variation among sites, but because the effect of size differences were accounted for in PC1 and PC2, that remaining variation was attributed to shape differences.

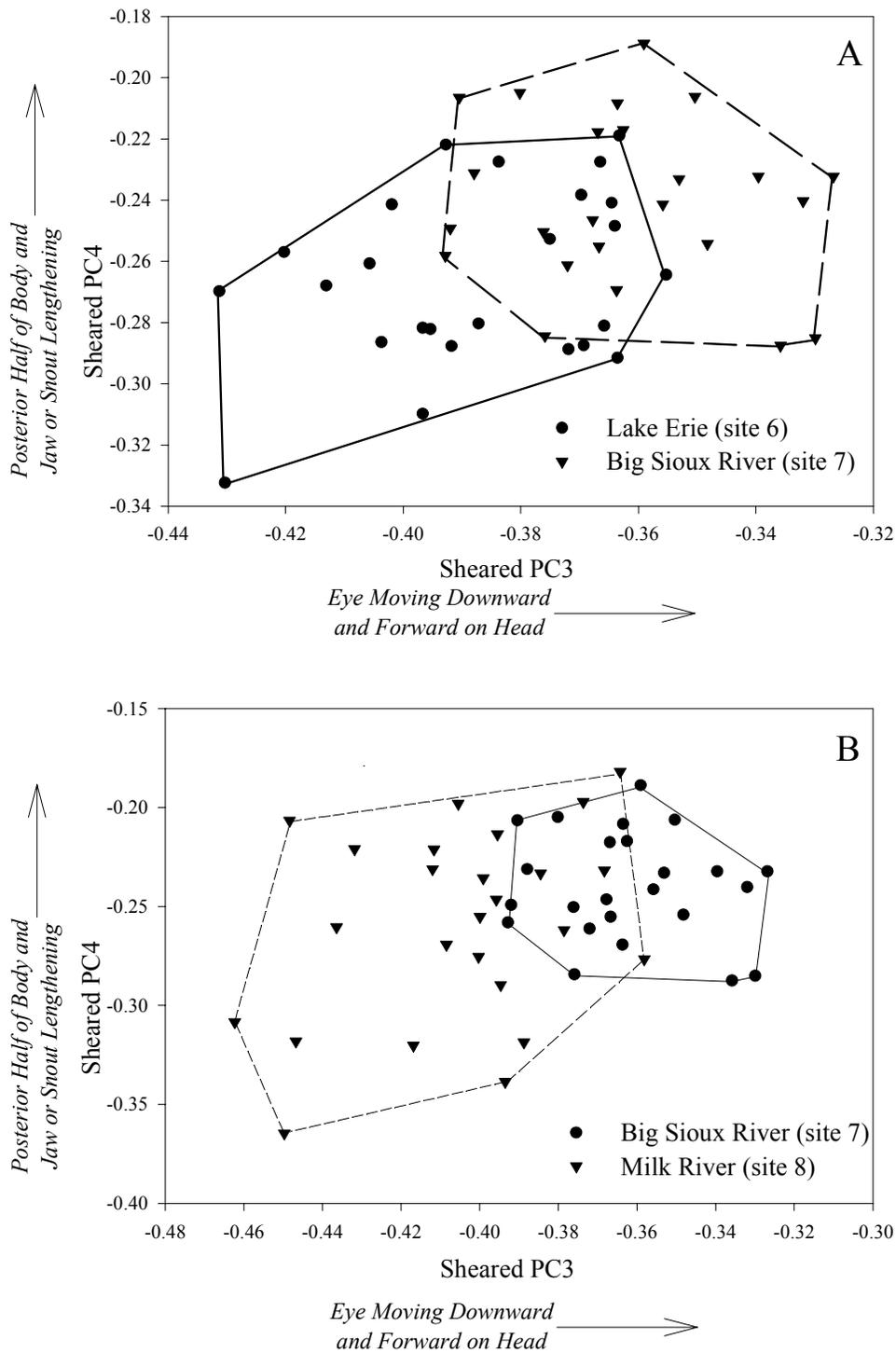


**Figure 4.** Location of truss elements with high eigenvector loadings. Solid lines are truss elements of sheared PC3 and dotted lines are truss elements of sheared PC4.

The plot of sheared PC3 on sheared PC4 showed that the variability within sites was greater than among sites (Figure 5). Overlap occurred among all sites, with only three sites showing any degree of visual discrimination. The overlap region between Lake Erie (site 6) and the Big Sioux River (site 7) contained less than half of the points from either site (Figure 6A). A MANOVA determined that these two sites significantly differed across both the sheared PC3 axis ( $P < 0.001$ ) and the sheared PC4 axis ( $P = 0.002$ ) thus making them significantly different clusters (Wilks'  $\Lambda$ :  $P < 0.001$ ). The proportion of incorrectly classified data using a DFA was 0.326. This value can be roughly interpreted as the proportion overlap between the two sites. Though not distinct clusters, the plot of these two sites indicated that Big Sioux River fish tend to have more anterior-ventral placed eyes and a lengthened posterior half of the body relative to Lake Erie individuals. The emerald shiners from the Milk River site were more variable than either the Big Sioux River or Lake Erie fish, yet they also showed some discrimination from the Big Sioux River fish (Figure 6B). A MANOVA for these two sites indicated that means were significantly different across sheared PC3 ( $P < 0.001$ ), but not across sheared PC4 ( $P = 0.101$ ). Despite the lack of discrimination across sheared PC4, enough overall separation was present to find that the two clusters were different from one another (Wilks'  $\Lambda$ :  $P < 0.001$ ).



**Figure 5.** Plot of size-free, sheared principal component scores for *Notropis atherinoides* from all sites.



**Figure 6.** Site separation along sheared PC3 and sheared PC4 for *Notropis atherinoides*. Polygons enclose all data from one site.

The DFA for these two sites determined that 0.203 of the data were incorrectly classified. Like the Lake Erie individuals, emerald shiners from the Milk River tended to have more dorsal-posterior placed eyes and a shorter length for the posterior half of the body.

Measurements for eye diameter, head depth at pupil, and mandibular jaw width were considerably less variable within sites than the PC scores of the box-truss network (Table 2). The transformed and length standardized values for eye, head, and jaw significantly differed between pairs of sites (Table 3). For eye diameter, Lake Francis Case (site 5) emerald shiners had the greatest eye diameter, while Yellowstone River fish had the smallest eye diameter. These two sites significantly differed from each other and from all other sites (Table 3). Head depth was lowest for the Milk River (site 8) and differed from all other sites. The inter-reservoir region (site 3) was the only other site that differed from others in head depth. It differed from the Yellowstone River (site 2), Lake Francis Case (site 5), and Lake Erie (site 6). Jaw width in the unimpacted Montana reach (site 1) differed from all sites except Lake Francis Case (site 5) while the remaining sites showed a total of six paired differences (Table 3). These three measurements provided more contrast among sites than the box truss network; however, they do not indicate consistent character interdependence.

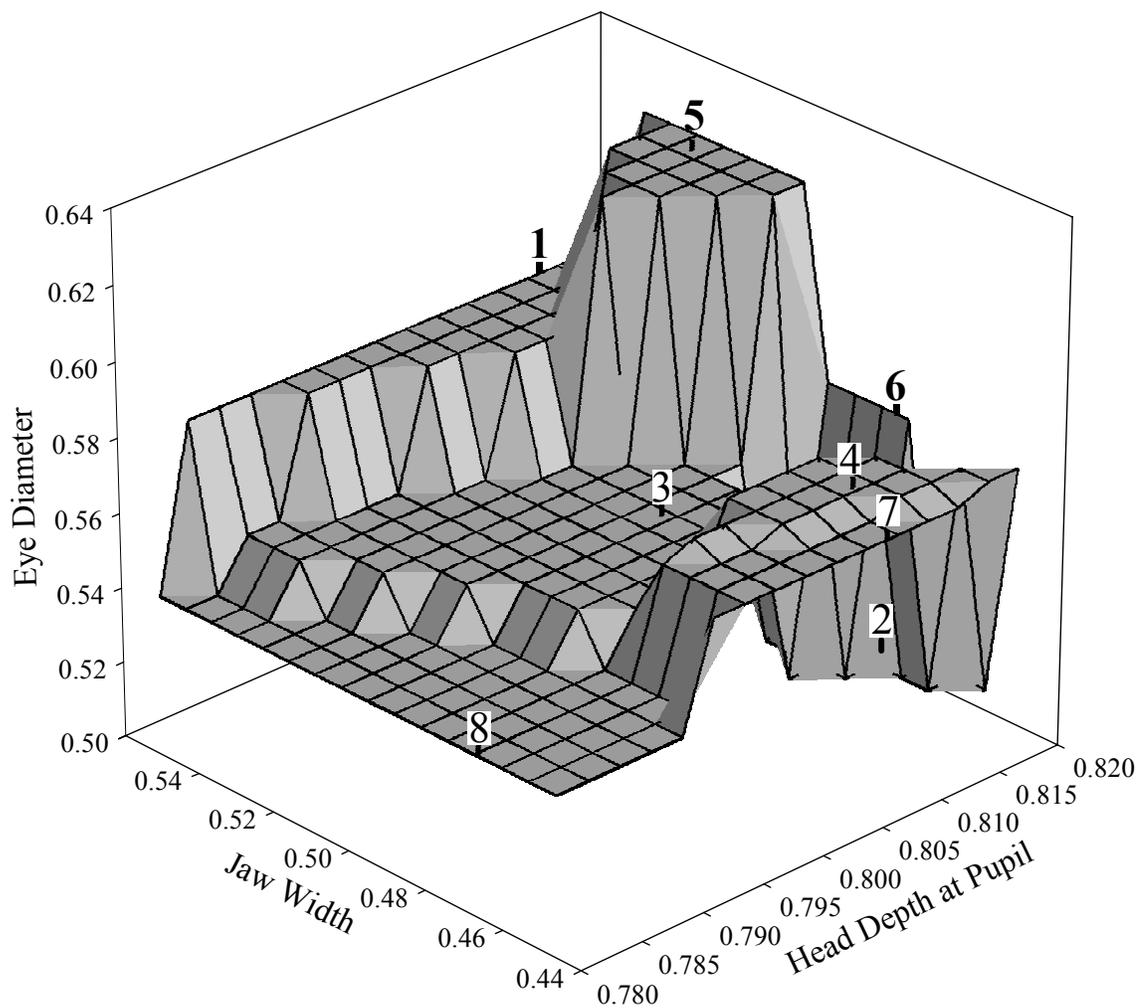
**Table 2.** Non-truss, morphometric data for *Notropis atherinoides* from each site. Site numbers refer to locations in Figure 1. Standard length (SL), eye, head, and jaw were measured in mm. Empirical data were used to calculate mean and standard error (Std. Err.), but are  $\log_{10}$  transformed and adjusted by length for analyses.

| Site | Measurement | N   | Mean   | Std. Err. |
|------|-------------|-----|--------|-----------|
| 1    | SL          |     | 60.926 | 1.380     |
|      | Eye         |     | 4.092  | 0.079     |
|      | Head        | 49  | 7.241  | 0.124     |
|      | Jaw         |     | 3.820  | 0.076     |
| 2    | SL          |     | 48.528 | 1.452     |
|      | Eye         |     | 3.080  | 0.089     |
|      | Head        | 25  | 6.108  | 0.189     |
|      | Jaw         |     | 2.924  | 0.096     |
| 3    | SL          |     | 55.925 | 1.387     |
|      | Eye         |     | 3.666  | 0.079     |
|      | Head        | 87  | 6.662  | 0.155     |
|      | Jaw         |     | 3.266  | 0.072     |
| 4    | SL          |     | 45.666 | 0.935     |
|      | Eye         |     | 3.419  | 0.038     |
|      | Head        | 149 | 5.689  | 0.108     |
|      | Jaw         |     | 2.742  | 0.050     |
| 5    | SL          |     | 55.040 | 0.723     |
|      | Eye         |     | 4.236  | 0.060     |
|      | Head        | 25  | 6.752  | 0.082     |
|      | Jaw         |     | 4.292  | 1.156     |
| 6    | SL          |     | 66.764 | 1.096     |
|      | Eye         |     | 4.152  | 0.071     |
|      | Head        | 25  | 7.964  | 0.149     |
|      | Jaw         |     | 3.368  | 0.073     |
| 7    | SL          |     | 61.933 | 0.489     |
|      | Eye         |     | 3.975  | 0.056     |
|      | Head        | 24  | 7.300  | 0.108     |
|      | Jaw         |     | 3.033  | 0.057     |
| 8    | SL          |     | 56.035 | 1.748     |
|      | Eye         |     | 3.558  | 0.105     |
|      | Head        | 26  | 6.319  | 0.206     |
|      | Jaw         |     | 3.085  | 0.144     |

**Table 3.** Significant differences in eye diameter, head depth at pupil, and jaw width between pairs of sites where *Notropis atherinoides* were collected. Sites were compared using an ANOVA for individual variables to produce the p-values below. The composite column contains p-values for Wilks'  $\Lambda$  in a MANOVA for all variables. The DFA Error is the proportion of variables incorrectly classified by cross-validation in a discriminant function analysis.

| Sites | Eye    | Head   | Jaw    | Composite | DFA Error |
|-------|--------|--------|--------|-----------|-----------|
| 1-2   | <0.001 | 0.366  | <0.001 | <0.001    | 0.152     |
| 1-3   | 0.005  | 0.137  | <0.001 | <0.001    | 0.373     |
| 1-4   | 0.144  | 0.288  | <0.001 | <0.001    | 0.198     |
| 1-5   | 0.003  | 0.411  | 0.4266 | 0.023     | 0.251     |
| 1-6   | 0.298  | 0.317  | <0.001 | <0.001    | 0.141     |
| 1-7   | 0.213  | 0.515  | <0.001 | <0.001    | 0.124     |
| 1-8   | 0.001  | <0.001 | <0.001 | <0.001    | 0.197     |
| 2-3   | 0.001  | 0.029  | 0.153  | <0.001    | 0.272     |
| 2-4   | <0.001 | 0.078  | 0.288  | <0.001    | 0.127     |
| 2-5   | <0.001 | 0.919  | 0.257  | <0.001    | 0.060     |
| 2-6   | <0.001 | 0.884  | 0.968  | <0.001    | 0.160     |
| 2-7   | <0.001 | 0.199  | 0.025  | <0.001    | 0.108     |
| 2-8   | 0.004  | <0.001 | 0.575  | <0.001    | 0.099     |
| 3-4   | 0.004  | 0.584  | <0.001 | <0.001    | 0.320     |
| 3-5   | <0.001 | 0.035  | 0.269  | <0.001    | 0.275     |
| 3-6   | 0.153  | 0.024  | 0.153  | 0.003     | 0.269     |
| 3-7   | 0.243  | 0.708  | 0.001  | <0.001    | 0.208     |
| 3-8   | 0.320  | <0.001 | 0.046  | 0.001     | 0.340     |
| 4-5   | <0.001 | 0.095  | 0.005  | <0.001    | 0.267     |
| 4-6   | 0.877  | 0.058  | 0.269  | 0.185     | 0.303     |
| 4-7   | 0.630  | 0.962  | 0.484  | 0.912     | 0.353     |
| 4-8   | <0.001 | <0.001 | 0.648  | <0.001    | 0.264     |
| 5-6   | <0.001 | 0.810  | 0.257  | <0.001    | 0.240     |
| 5-7   | <0.001 | 0.213  | 0.072  | <0.001    | 0.183     |
| 5-8   | <0.001 | <0.001 | 0.179  | <0.001    | 0.139     |
| 6-7   | 0.688  | 0.197  | 0.010  | 0.071     | 0.429     |
| 6-8   | 0.001  | <0.001 | 0.532  | <0.001    | 0.234     |
| 7-8   | 0.004  | 0.006  | 0.256  | 0.001     | 0.230     |

A simultaneous scatter plot of eye diameter, head depth, and jaw width produced distinct composite morphotypes that revealed patterns among sites (Figure 7). The Lake Francis Case (site 5) emerald shiners had relatively high eye, head and jaw values, while the Milk River (site 8) fish had relatively low values for all three measurements. The channelized (site 4), Lake Erie (site 6), and Big Sioux River (site 7) fish formed an apparent group with similar values for all three measurements (Figure 7). A MANOVA on all three variables determined that sites 4, 6, and 7 were the only sites that were not significantly different from one another (Table 3). All remaining sites had sufficient variation in one or more of the variables to make them each significantly different from all other sites. The DFA then determined that the proportion of incorrectly classified data for each site pair comparison ranged from 0.060 to 0.429 with 71% of data falling between 0.100 and 0.300. These values can be roughly interpreted as the proportion of 3-dimensional overlap between two sites regardless of mean differences.



**Figure 7.** Inter-relationship between eye diameter, jaw width, and head depth at pupil for *Notropis atherinoides* at the eight sites. Site locations are shown in Figure 1.

## DISCUSSION

The objective of this study was to determine whether habitat alteration and fragmentation in the Missouri River affected the morphology of a native species. The habitat of much of the Missouri River has been changed from lotic to lentic and from wide floodplain to channelized. These habitat changes created an environment where species were forced to adapt to altered conditions. I assessed numerous shape characteristics to determine if river alteration had produced new morpho-types among the habitats.

Several consistent trends among sites appeared in both box-truss and non-truss measurements. Lake Erie and the Big Sioux River appeared to consistently be distinguished from the other sites because of their smaller degree of variation among individuals. The data from both sites overlapped all other data to some degree, but they did not show the wide variation seen within the other sites. The mean shape of the Lake Erie fish was significantly different from only the Big Sioux River site. However, the three non-truss measurements on the head distinguished the Lake Erie fish as different from all sites except the Big Sioux River and channelized sites. These two opposing results between Lake Erie and the Big Sioux River show that morphometric characteristics can vary independently of one another. Therefore, there is no idealized form that either expands or contracts equally, instead there are individual sub-components of form whose change depends on local conditions or random drift rather than a conformity to isometric or allometric growth.

Emerald shiners collected from the mainstem Missouri River showed no mean

shape differences among sites. However, all mainstem Missouri River sites differed from each other for the composite non-truss measurements. Again, this supports the concept of specific morphometric changes occurring irrespective of overall shape. The box-truss shape data revealed large variation within each mainstem Missouri River site that was not related to sample size or site length. That large variation cannot be a result of habitat alteration, because increased variation would not be distributed equally among all sites. Most likely, the emerald shiners of the mainstem Missouri River possessed inherently high variation in morpho-type prior to any habitat alteration.

The differences in the non-truss measurements suggest several relationships. Emerald shiners from Lake Francis Case (site 5), the only lentic site sampled in the Missouri River, had larger eye diameters than did fish from other sites, but Lake Erie emerald shiners had eye diameters similar to those of other mainstem Missouri River fish. In a lentic habitat, with lower turbidity (2-10 NTU) than the river habitats (10-300 NTU), Lake Francis Case and Lake Erie individuals feed more on plankton than periphyton (Fuchs 1967; Young et al. 1997). Therefore, Lake Francis Case fish may have shifted to larger eyes to aid in their new more sight-dependent feeding mode (Taylor and Bentzen 1993; Pankhurst 1989). In contrast, the fish from the Yellowstone River and Milk River show an opposite response in eye diameter. Fish from these two sites had the lowest eye diameters and come from two sites that have greater mean turbidities ( $\bar{x} > 100$  NTU) than the mainstem Missouri River ( $\bar{x} < 80$  NTU, Young et al. 1997). The Milk River is named for its turbid appearance and the Yellowstone River is noted for contributing muddy flows at its confluence with the Missouri. In these habitats, the local fish may have

shifted to smaller eye diameters as their use became of less importance in low visibility habitat. While shape is usually attributed to velocity and swimming behavior, eye diameter may reflect the relative dependence on vision.

Head depth and jaw width greatly distinguished fish among sites as well, but ascribing ecological significance to these relationships was more difficult. The same two turbid rivers that shared the characteristic small eye diameters were extreme opposites in head depth. The Yellowstone River fell in range with most other sites, but the Milk River had a much narrower head depth than the other sites. Typically, this could imply a hydrodynamic adaptation to higher velocities, but without detailed hydrologic data from the Milk River, any explanation would be purely speculative. Variation in jaw width among sites was more evenly distributed, but interestingly, the four highest mean jaw widths were from the four sites with the lowest turbidities. The upper unimpacted site is mostly mountain runoff over coarse substrate, the inter-reservoir site receives its water from clear reservoirs in which sediment has settled out, and the Lake Francis Case and Lake Erie sites are free from continuous flow that suspends sediment. This relationship was not as strong as the eye diameter results, but does suggest that perhaps in these habitats where visibility was greater, the assumed more discriminate selection of food may have lead to a shift in wider jaws to accommodate larger prey. Like eye diameter, it seems that these two measurements are also independent of overall shape and respond to more specific habitat attributes rather than a composite form needed for swimming ability.

The absence of shape differences among the Missouri River sites can be explained

by two possible scenarios. First, the Missouri River may provide sufficiently variable microhabitats within each site that are common among sites. Thus, emerald shiners along the entire Missouri River may be able to find similar patches of habitat amenable to their needs. Selective pressures and habitat conditions then would not substantially differ among sites. Therefore, Missouri River emerald shiners can persist as they historically did with little influence from large scale habitat alterations. Watts et al. (1995) also suggested this phenomenon to explain why genetically distinct and sympatric populations of *Galaxias occidentalis* failed to show distinct morphological differences. Second, the high variability among all Missouri River sites may be an inherent artifact of the historic Missouri River. The historic Missouri River was more hydrologically unpredictable than it is today. Those conditions may have produced a species with a large norm of reaction, capable of tolerating a large range of conditions. Whether it be turbidity, velocity, diet, or other highly variable environmental conditions, the wide range of phenotypic expression would allow the species to persist. The two lentic sites and the Big Sioux River had less variability in shape. The two lentic sites obviously have a more stable environment than the mainstem Missouri River sites. In those cases, there may not be as great a need for phenotypic variability. Perhaps the norm of reaction was lower there because it simply could be when more constant and environmentally stable conditions exist. This second hypothesis is supported by Poff and Ward (1989) who link communities of ecological generalists to highly variable hydrologic conditions and specialists to more benign and predictable conditions. Thus populations of emerald shiners with highly variable morphotypes may be adapting spatially to physical habitat and temporally to hydrologic

conditions.

With little change in overall shape, but many differences in specific characteristics, it is clear that emerald shiners phenotypically respond to local conditions through focused and independent character shifts. It appears that environmental conditions create selective pressures that affect body shape were insufficient among sites to cause significant shifts within the reaction norm. However, the conditions that affect eye size, head depth, and jaw width, did seem sufficient to produce distinct characteristic differences among many sites. The specific differences seen cannot be necessarily attributed to habitat alteration because it is unknown whether these differences were present prior to changes. Only the large eyes of Lake Francis Case fish suggest that impoundment may have favored a phenotypic shift.

The documented high degree of plasticity in emerald shiners was confirmed by this study. The high variability was likely the result of high reaction norms developed from historic variable habitat conditions and over time, incorporated into the genetic architecture. Possessing this phenotypic plasticity while only showing changes in a portion of all morphometric characteristics, reveals how local habitat conditions can produce specific phenotypic shifts in some characteristics while maintaining overall natural variability in other morphometric attributes.

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## Chapter 5

Performance testing of emerald shiners (*Notropis atherinoides*)  
from adjacent reservoir and river habitats

## ABSTRACT

Emerald shiners (*Notropis atherinoides*) from a reservoir and adjacent river were used to detect whether habitat alteration had caused differences in swimming ability. I collected fish from Lewis and Clark Lake (reservoir) and the adjacent Missouri River below the dam in South Dakota. The two groups were once part of a continuous population, but are now separated by a dam with one group living in lentic habitat and the other in lotic. The fish were acclimated to laboratory conditions, then forced to swim at increasing velocities in a flow chamber. Velocities were increased by 11.1 cm/s every 5 minutes until fish were fatigued and unable to swim. Neither length, weight, nor condition differed between the two groups allowing direct comparisons of swimming performance. Despite habitat, diet, and morphometric differences between the river and reservoir groups, swimming performance did not significantly differ ( $P=0.431$ ). There was a notable behavioral response in both groups where 45% of reservoir and 50% of

river individuals consistently fatigued within one minute after a change in velocity. This may suggest an ability to endure constant velocity conditions for extended periods, but a tendency to succumb to abrupt changes in velocity. The performance data were used as a measure of overall swimming ability, but if there are habitat-related changes between the reservoir and river fish, they may only be detectable for specific characteristics that do not significantly affect overall performance.

## INTRODUCTION

When a species exists in different habitat types, it will adapt to the local conditions (Riddell and Leggett 1981; Vondracek et al. 1982; Swain and Holtby 1989; McLaughlin and Grant 1994; Bisson et al. 1998). In natural continuous habitats, conditions gradually differ across the landscape, but anthropogenic habitat alteration can produce conditions where habitats change suddenly and sharply. In the Missouri River, 1,095 km of the 3,500 km of historically riverine habitat have been impounded in reservoirs. The physical, chemical, and biological properties that characterize the resulting lentic and lotic habitats have substantially diverged from historic conditions (Hesse et al. 1989; Schmulbach et al. 1992). Those changes in temperature, velocity, hydrology, turbidity, nutrient availability, prey base, and predation have created selective pressures that may result in physiological, morphological, and behavioral changes between emerald shiners from the two different habitats (Hubbs 1941; Bams 1967; Riddell and Leggett 1981; Chiasson 1993; Law and Blake 1996).

Emerald shiners are an abundant species native to the Missouri River and are

found in both reservoir and river habitats. They are generalists that have a broad potential niche (Scott and Crossman 1973) thus enabling them to adapt to diverse new habitat types. Adaptation to new and contrasting habitats may then expose character differences between the reservoir and river fish. Riverine fish may have different muscle mass, body shape, or condition than fish from a reservoir habitat causing differences in performance (Meyer-Rochow and Ingram 1993; Broughton et al. 1981). In Chapter 4, the body shape comparisons between fish from these habitats found the river fish to have a greater variability in body shape than reservoir fish. The more shape-variable group of river fish contained individuals with both broader and more elongated body forms than the lake fish. Those different body forms could potentially provide hydrodynamic performance advantages. The river and reservoir fish have different diets (Fuchs 1967) that may affect metabolism and thus their aerobic stamina. Stamina should not be affected by the proportion of males or females within or between the river and reservoir habitats because emerald shiners do not display sexually dimorphic forms or observable gender specific behavioral differences except during spawning season (Flittner 1964). These contrasts in morphology, physiology, and ethology between the river and reservoir groups may be sufficient to cause detectable performance differences.

Performance testing has most often been used to compare hatchery versus wild fish (Bams 1967; Horak 1972; McNeish and Hatch 1978; Lagasse et al. 1980), toxicity effects on fish (Waiwood and Beamish 1978; Buckley et al. 1985; Hamilton et al. 2000), and the effect of size on swimming (Webb 1978; Berry and Pimental 1985; Mourad 1991). These performance data were typically used to establish suitable habitat or rearing

criteria that would accommodate a particular species. The role of habitat in determining swimming performance has been much less studied (Taylor and Foote 1991). This study was designed to detect differences in swimming performance between fish from different habitat types to determine if habitat alteration produces changes in swimming ability.

## METHODS

Emerald shiners were collected using a 9.14-m bag seine from two sites on the Missouri River in South Dakota: a reservoir site located in Lewis and Clark Lake 22.4 km above the dam (river km 1325) and a river site 34.3 km below the dam (river km 1269). Emerald shiners were collected from the reservoir site on 13 September 1999 and from the river site on 8 October 1999. Fish were then put in an aerated hauling tank filled with water from each site and treated with NaCl and nitrofurazone antibiotic. They were then transported 2.5 hours to the lab where they were kept in a 1500-L, aerated, flow-through tank. Temperature was maintained at 16°C for 14 to 18 days before testing and the fish were fed TetraMin<sup>®</sup> flake food twice daily. Water was dechlorinated using sodium thio-sulfonate, and treated with nitrofurazone to control diseases.

The swimming performance chamber was a plexiglass tube with an inside diameter of 8.5 cm. Flow was produced by a 1,119-J/s electric pump. An in-line flow meter provided a digital measure of discharge in liters/second (L/s). Tube diameter and discharge were used to determine that 0.631 L/s equated to a velocity of 11.1 cm/s inside the performance chamber. Water was recirculated through a 380-L supply tank where temperature was maintained with a chiller at 16°C.

I tested 66 lake fish and 36 river fish that ranged from 55.4 to 83.7 mm (TL). Fish were tested alone and only once. They were sealed in the chamber and subjected to 5-minute cycles at increasing velocities (Table 1). The increase in velocity between cycles was immediate. Swimming was timed from the beginning of flow until the fish was impinged against the end of the chamber. Fish that did not complete 3 full cycles (table 1) were considered defective in health or ability and excluded from analyses. After being removed from the chamber, fish were weighed to the nearest 0.01 g and measured with digital calipers for standard (SL) and total length (TL) to the nearest 0.1 mm. Tail length was calculated as (TL - SL), Critical swimming velocity ( $U_{crit}$ ) was calculated with the following formula:  $U_{crit} = V_F + V_C(T/D)$  where  $V_F$  = velocity (cm/s) in chamber during last completed cycle,  $V_C$  = increase in velocity per cycle,  $T$  = time swam during failure cycle, and  $D$  = duration of each interval. Relative condition factors were calculated for all fish using  $K_n$  by calculating a pooled weight-length relationship for each group (Le Cren, 1951). Physical data between the lake and river fish were not normally distributed. Differences were tested using Mann-Whitney  $U$  tests and correlations between performance and size were calculated using a Spearman test.

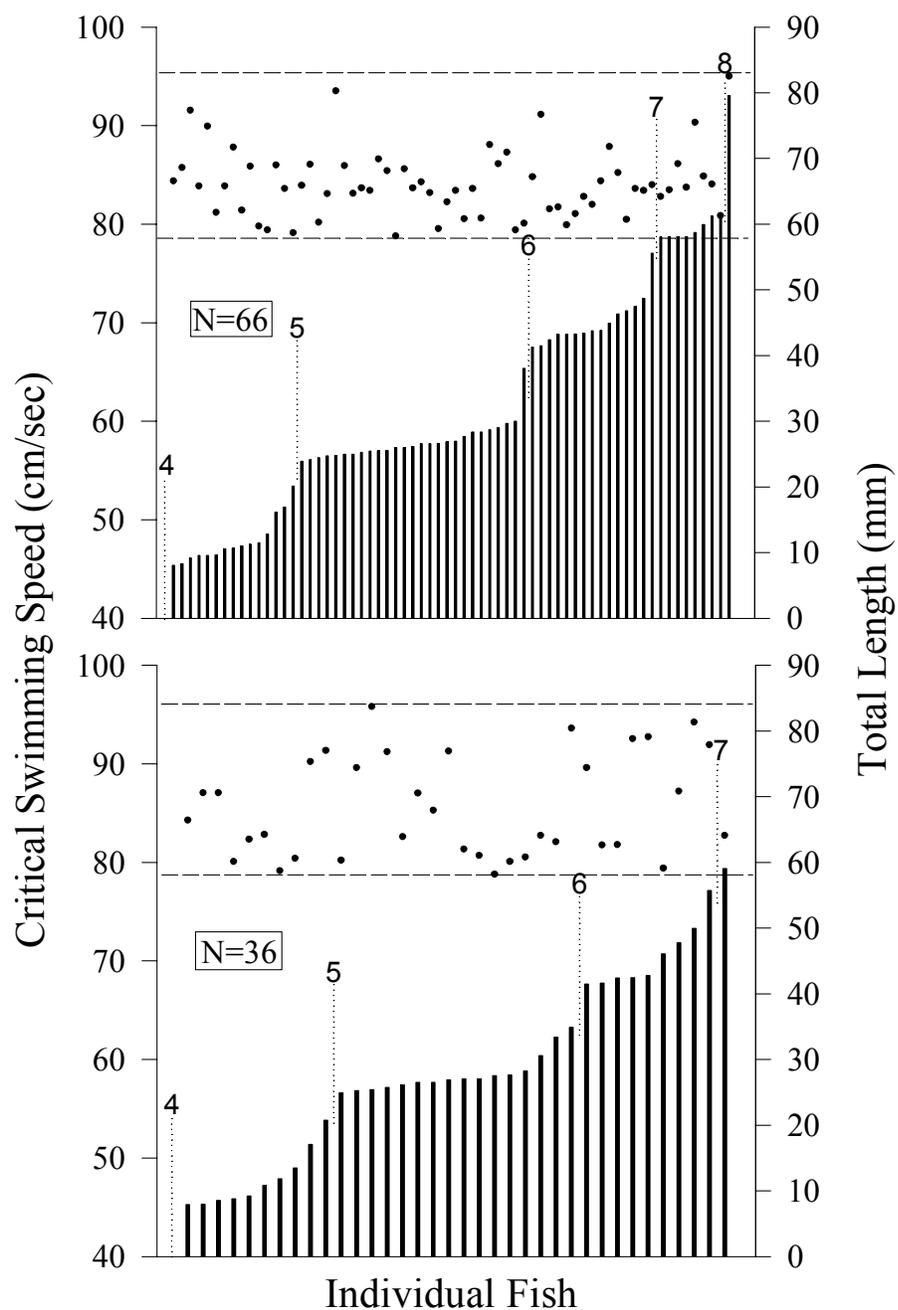
**Table 1.** Stepped flow scheme intervals for performance testing.

| Cycle number         | 1    | 2    | 3    | 4    | 5    | 6    | 7    | 8    |
|----------------------|------|------|------|------|------|------|------|------|
| Cycle duration (min) | 5    | 5    | 5    | 5    | 5    | 5    | 5    | 5    |
| Discharge (L/s)      | 0.6  | 1.3  | 1.9  | 2.5  | 3.2  | 3.8  | 4.4  | 5.0  |
| Velocity (cm/s)      | 11.1 | 22.2 | 33.3 | 44.4 | 55.5 | 66.6 | 77.7 | 88.8 |

## RESULTS

The reservoir fish had individuals that swam from cycle 4 to cycle 8. The river fish ranged from cycle 4 to cycle 7. For both reservoir and river fish, cycle 5 exhausted more fish than any other cycle. Performance was unaffected by individual differences within each group. The only significant correlation between performance and length, weight, or condition data was  $U_{crit}$  and  $Kn$  in the lake fish ( $r_s=0.285$ ,  $P=0.020$ ). This weak correlation and the absence of any other correlations between individual characteristics and performance data indicated that covariate data did not exist. Based on that premise, I tested  $U_{crit}$  between groups directly with no size correction needed (Figure 1) and detected no difference ( $P=0.431$ ) between lake ( $\bar{x} = 61.5 \text{ cm/s} \pm 11.2$ ) and river ( $\bar{x} = 59.1 \text{ cm/s} \pm 9.4$ ) fish. Of the variables  $U_{crit}$ , TL, SL, weight,  $Kn$ , and tail length, only tail length was significantly different ( $P<0.001$ ) between the lake ( $\bar{x} = 11.4 \text{ mm}$ ) and river ( $\bar{x} = 12.5 \text{ mm}$ ) fish. Distributions of swimming performance data between the two groups were not statistically different either (Kolmogorov-Smirnov:  $P=0.442$ ).

Lake and river fish both tended to cease swimming soon after the 11.1 cm/s increases in velocity. Within the first minute (20% of a cycle duration) of a change in velocity, 45% of lake fish and 50% of river fish were impinged. By the end of the second minute of a cycle (40% of a cycle duration), 86% of the lake fish and 72% of the river fish were impinged. These data showed a stair-stepped pattern of fatigue after a change in velocity among the majority of fish (Figure 1).



**Figure 1.** Critical swimming speeds and associated lengths of emerald shiners from reservoir and river habitats. Numbered, vertical dotted lines indicated changes in cycle number (Table 1) during the stamina tests. Horizontal dashed lines indicate the range of lengths.

## DISCUSSION

The results for river swimming ability agree with those from other studies, but there have been no swimming studies done on reservoir or lentic emerald shiners. The river fish data match those of Tunink (1977) who found that emerald shiners (34-48 mm SL) had a mean critical swimming velocity of  $53.26 \text{ cm/s} \pm 7.16$ . His fish were collected from the same river reach that I sampled, but were tested immediately after capture on site. Another study on the Mackenzie River, Canada found a mean critical swimming velocity of 59 cm/s for emerald shiners having a mean fork length of 65 mm (Jones et al. 1974).

The reservoir and river fish were subjected to identical forced swimming conditions. The variables that had the potential for obscuring the results did not significantly differ between the reservoir and river fish, thus confirming that physical size did not influence the results. The absence of correlation between size and swimming performance suggests that all the fish fell into a size range narrow enough to negate any potential size-specific advantages. After controlling the experimental environment and any effects of size within or between the two groups, I found that swimming endurance did not differ between the reservoir and river fish. Thus, the absence of performance differences reflected the apparent inadequacy of habitat or variable morphometry to produce differential performance abilities. The lack of significantly different distributions between reservoir and river swimming data indicated there were no shape-specific differential swimming abilities related to the morphometric shape distributions. In fact, the swimming performance variation was greater for reservoir fish while their

shape was less variable than river fish. Although these two groups of fish have inhabited adjacent, yet very different habitats for the past 40 years, they seem to have adapted in ways that do not significantly affect their swimming ability.

I noticed a pattern in the data that may provide information useful in assessing velocity tolerances for many similar species. The data showed that the majority of fish became fatigued soon after the velocity change in their final cycle. This pattern occurred at each velocity interval in which fish were fatigued. Very few individuals reached fatigue during the third, fourth, or fifth minute of a cycle. This suggests that there is a stamina threshold. If an individual can sustain its swimming and endure the first 2 minutes of a cycle, it will likely complete the remaining 3 minutes as well. This observation may have implications for the design of structures that affect aquatic habitat (Berry and Pimentel 1985). If culverts, bridge bases, and other structures are designed to create gradual rather than sudden velocity increases, they may enable fish to tolerate an increased velocity for a much longer period and ease fish passage stress.

The absence of intraspecific differences between the reservoir and river fish leads to two possible conclusions. First, the two groups have not become different in swimming ability from one another in any measurable way. This would mean that the two new habitats, though very different, do not produce selective pressures sufficient to cause divergent local swimming adaptations. If the emerald shiners can find similar available habitat in either the reservoir or river, then they may be using similar microhabitat portions within the dissimilar macrohabitats. Second, differences in physiology, morphology, and ethology may differ between the reservoir and river fish, but

those differences may not be sufficient to provide a swimming advantage. Zimmerman and Richmond (1981) measured increased heterozygosity in the malate dehydrogenase locus for *Notropis lutrensis*. Individuals residing in the thermally variant river below the more stable reservoir responded by producing polymorphisms. The two groups became genetically identifiable by their various alleles, but an underlying or subtle adaptation such as this does not necessarily translate into a performance advantage (Berry and Hudy 1983).

Additionally, inherent differences among individuals may mask any variation arising from measured characteristic changes. I used swimming performance tests to evaluate the sum effect of biological and environmental changes on reservoir and river emerald shiners. However, it is likely that the sum effect includes multiple variables that produce immeasurable interactions. These variables and interactions likely cause as much individual as group variation in stamina and may even negate or mask specific differences. Therefore, measurements of specific characteristics and how they differ between the reservoir and river fishes may provide a more accurate assessment of changes in response to habitat alteration.

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## Chapter 6

### Conclusions

The objectives of this study were to 1) determine whether dams and impoundments have genetically isolated populations, 2) determine the role that habitat perturbation may play in physically shaping a species, and 3) determine if stamina is related to body shape and affected by flow velocity. I designed the preceding four studies to not only specifically address these objectives, but to also provide a comprehensive assessment of a species responses to large scale habitat alteration. The intraspecific character variation and population structure data were interesting studies, but they also provide biological information to the agencies responsible for the management and regulation of the Missouri River.

The genetic population structure study was done to establish criteria on which the other three studies depended. Genetic population structure at its core identifies whether potential populations are isolated or interbreeding. For this study, the null hypothesis of genetic panmixia unfortunately has two explanations. First, the entire river may be completely panmictic and interbreeding. This is not likely because of the dams. Second, the Missouri River does have effectively isolated populations, but they have not had

enough time for random genetic drift to reveal allelic frequency changes. This is likely, but impossible to substantiate at this time. Rejecting the null hypothesis in this study would have clearly identified genetically distinct sub-populations, but failing to reject does not provide clear information on population structure.

Regardless of the true genetic population structure, I at least identified that the Missouri River fish I was studying were most likely all part of a single large continuous population. From that base, I could then make clear distinctions about the mechanisms responsible for differences seen in the other studies. Genetic drift and isolation could be ruled out as contributing to differences among sites. Therefore, intraspecific variation in physical characteristics either developed independently from genetics or a historically diverse genetic architecture allowed for the variation. In either case, intraspecific variation must have been the result of phenotypic plasticity, not population specific divergence.

The meristics study was designed to determine whether alterations to the hydrology of the Missouri River were capable of disrupting natural changes in meristic counts. Differences in meristic counts are common and expected and usually a function of environmental conditions. I made the meristic counts along the length of the Missouri River to find whether the areas of alteration had caused a deviation from what was expected to be natural increase, decrease, or stasis. The results of this study were difficult to interpret. Three out of the five characteristics that were counted showed no difference among sites. Pectoral fin rays differed among sites and showed an anomalous peaking in number around the impounded areas of the river. Both the far upper and far lower parts

of the river then decreased similarly. The vertebrae data followed a classic pattern of increasing linearly with latitude. Independent meristic changes are not uncommon, but they do not provide conclusive evidence for determining the cause of the differences.

Meristic counts are controlled by both genetics and the environment. The genetics study already established that the fish were not definitively dissimilar, so differences are likely the result of environmental variation. Meristic characters are formed during the early developmental period of the fish and only reflect hydrologic and water quality conditions during that 2-month period. That same time of the late spring and summer is when nearly all Missouri River fish spawn. This knowledge of emerald shiner larval development may be applicable to other species to help explain how reproduction and development are affected by altered conditions in different parts of the Missouri River.

The analysis of shape and morphology was done to identify whether a known morphologically plastic species had developed divergent morphotypes to adapt to altered habitat conditions. The intuitive hypothesis was that fish in high velocity habitats would have more fusiform shapes while fish in slow or lentic waters would be more robust. This had been reported in the literature several times where supposed lake and river subspecies of emerald shiners existed. The morphometric measurements that were used to propose that classification were done 25-100 years ago and were rather simplistic. Lengths of the two groups of fish differ substantially with the lake fish being much larger. When log transformed standard lengths are used as a common denominator for all measurements, the effects of size are not completely removed. The early systematists were not aware of more advanced techniques that would appear decades later and be able

to truly standardize the data. After using the box-truss protocol, I was able to find that differences among sites throughout the Missouri River Basin and Lake Erie were negligible. The differences were not in actual form, but in the individual variability within sites. Differences in mean shape only differed among sites with a narrow variation in form. All mainstem Missouri River sites had high variability and contained individuals with more extreme shape differences. However, the extremes evenly spanned the same means. Therefore, the most conclusive information from the shape analysis was that variability in shape was higher among mainstem Missouri River sites than in lentic and tributary sites.

The additional morphometric data provided some more specific details about the physical characteristics of the fish. The eye, head, and jaw results showed differences among most sites for at least one characteristic. I speculated on the ecological significance of the differences as they pertained to local habitat conditions, but controlled testing would be needed to verify those hypotheses. The independent differences among the three measurements were consistent with the type of response seen in the meristic data. Eye, head, and jaw data did not exhibit any covariance or other relationships. This led to the conclusion that local habitat conditions caused specific changes in characteristics while not necessarily affecting other characteristics. These types of changes are the result of phenotypic plasticity and do not signify the existence of sub-populations, but they do indicate that distinctive forms may arise that are specifically adapted for a habitat type. Despite their population structure, these morphologic forms may constitute recognition as evolutionarily significant units (Chapter 2) and require

specific habitat characteristics conducive to their morphology. Emerald shiners are likely plastic enough to adapt to a variety of habitats, but other species that have a less variable morphology and are dependent on specific physical characteristics may be harmed by sudden habitat changes. I cannot definitively say that river alteration has produced specific new morphotypes or characteristics in emerald shiners, but I can identify differences in specific characteristics that appear to be a function of habitat and likely provide some type of competitive advantage.

The performance testing experiment was proposed and designed prior to obtaining the results from the morphology analyses. I had assumed that there would be a marked contrast in shape between reservoir and river fish. The experiment was meant to test whether the differences in body shape provided a hydrodynamic swimming advantage to one group or the other. However, the morphology results revealed that there was not a mean difference in shape between the two groups, there was only a difference in the amount of shape variation between the two groups. The experiment then became a test of whether a lacustrine or riverine life history provided a swimming advantage either as a result of muscle training or behavioral experience. The data showed random results suggesting that swimming ability in emerald shiners had not been even minimally affected by alteration of the Missouri River. As stated in Chapter 5, I believe that despite the changes in habitat, emerald shiners are likely able to find static waters throughout the mainstem Missouri River that would function the same as a reservoir environment. There are many other differences between the two groups such as diet, reproduction, and growth that were affected by habitat alteration, but swimming ability was not affected by the

changes.

In Chapter 1, I stated that the intraspecific variation among sites in the Missouri River would follow either a uniform, random, grouped, or graded pattern. For the mainstem Missouri River, genetic population structure followed the uniform pattern showing no appreciable differences in allelic frequencies. The meristic data were either uniform (dorsal, anal, and pelvic fins), grouped (pectoral fin), or graded (vertebrae). Box-truss data were uniform for the mainstem Missouri River, but grouped among some tributary and lentic sites. The other morphology data were grouped for some characteristics and sites, but random among others. Performance data were random among individuals and uniform between sites. It is apparent from these combined analyses that there is no covariance in characteristic changes for emerald shiners. The analyses also revealed that the form and life history of emerald shiners is affected at different scales. While specific morphometric measurements showed changes between neighboring sites, genetic analyses found no difference among all the mainstem Missouri River sites. It is clear that local environmental conditions affect some characteristics making it possible to differentiate them among sites, but determining the rate and degree of those changes will have to be done in the future now that a benchmark exists for current characteristics.

My studies were not intended to provide detailed management information. Aside from the difficulty of trying to manage species in an open system like the Missouri River, instituting any kind of specific plans would require facing tremendous political confrontations. Instead, I studied the effects that recent hydrologic management plans

have had on a species. The emerald shiner was used as an indicator species to gauge the biological effects of known physical habitat changes. Through this process I have found that the impoundments and channelization have had little effect on the genetic population structure, development, morphology, or swimming ability of this species.

Habitat changes in the Missouri River have certainly occurred, but the inherent variability of the emerald shiner appears to have provided it with the ability to adapt to numerous types of environmental conditions. However, the emerald shiner is not representative of all species in the Missouri River and should not serve as an indication of how all other species are responding to habitat alteration. Less phenotypically plastic species may have been much more adversely affected by the differing selective pressures caused by habitat alteration. By evaluating characteristics similar to those that I have used, managers and biologists may be able to identify specific adaptations that enable the persistence of a species or determine which environmental conditions are commensurate with specific morphological characteristics. Different species adapt to new conditions in different ways, so understanding the types, rates, and degrees of changes that occur in a species will provide the information necessary to assess its compatibility with its local habitat.