ECOLOGY AND EVOLUTIONARY BIOLOGY OF PHENOTYPIC PLASTICITY IN THE THREESPINE STICKLEBACK (GASTEROSTEUS SPP.)

by

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ABSTRACT

This work addresses questions concerning the evolution of diet-induced plasticity of trophic morphology in two species of freshwater threespine stickleback (Gasterosteus spp.). In chapter one I describe an experiment designed to answer the following questions about diet-induced morphological plasticity in these fish: (1) do the study species exhibit diet-induced morphological plasticity, and is this plasticity likely to be adaptive, (2) are different selective regimes associated with different degrees of plasticity, and (3) is there genetic variation for phenotypic plasticity in contemporary populations. The experiment revealed that these species exhibit plasticity that appears to be adaptive, and that an association exists between diet variability and the degree of diet-induced morphological plasticity as predicted by theory. It also revealed that genetic variation for morphological plasticity exists in both species.

Chapter two presents a second experiment designed to further explore the possibility that diet variability can drive the evolution of morphological plasticity. This experiment also had three objectives: (1) to quantify the time scale of morphological change to determine if it is compatible with that of natural diet variability, (2) to explicitly examine the adaptive significance of diet-induced morphological plasticity by measuring its effect on foraging efficiency, and (3) to examine the effect that short-term learning (behavioural plasticity) has on foraging efficiency and compare its importance to that of morphological plasticity. This second experiment revealed that the time scale of plastic change is roughly compatible with that of diet variability and that diet-induced morphological changes result in changes in foraging efficiency. It also revealed that behavioural plasticity affects foraging efficiency but that it affects a different component of the prey ingestion process than does morphological plasticity.

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GENERAL INTRODUCTION

Phenotypic plasticity is defined as repeatable environmentally-induced phenotypic change which occurs within an organism's lifetime (Bradshaw 1965; Stearns 1989; Scheiner 1993a). The functional relationship between the environmental factor of interest and the phenotype is described by a 'norm of reaction' (Suzuki et al. 1989). Although the significance of phenotypic plasticity was recognized years ago (Wright 1931; Schmalhausen 1949; Bradshaw 1965), phenotypic plasticity was not formally incorporated into evolutionary theory until quite recently (Levins 1968; Via and Lande 1985; Gomulkiewicz and Kirkpatrick 1992; Leon 1993; Gavrilets and Scheiner 1993a, 1993b). Consequently, empirical research in this field has undergone a recent increase as well (Schlicting and Levin 1986; Dodson 1989; van Noordwijk 1989; Witte et al. 1990; Wimberger 1991, 1992).

This thesis addresses questions concerning the evolution of diet-induced plasticity of the trophic morphology of two sympatric species of threespine stickleback (Gasterosteus spp.). The first chapter introduces the two study species and briefly reviews some relevant natural history. Here, I also describe an experiment designed to answer some fundamental questions regarding diet-induced morphological plasticity in sticklebacks. These include: (1) do these species exhibit diet-induced morphological plasticity, and if so, is the plasticity likely to be adaptive, (2) do the two species exhibit different degrees of plasticity as predicted by theory based on differences in their diet variability, and (3) is there genetic variation for phenotypic plasticity present in contemporary populations? As a subsidiary question I also asked: what proportion of the

morphological difference between the species might result from phenotypic plasticity? To answer these questions I compared the diet-induced morphological plasticity of these two recently diverged stickleback species.

This experiment is the first step towards testing theory concerning the evolution of morphological plasticity in wild populations. If diet-induced morphological changes are adaptive and if the species that normally experiences high diet variability also exhibits a greater degree of plasticity, then this would suggest that diet variability may be important in the evolution of trophic morphological plasticity. The presence of genetic variability for morphological plasticity would strengthen this claim. Such results would also lend support to recent theoretical studies of the evolution of phenotypic plasticity in general.

Chapter two presents a second experiment designed to further explore the possibility that diet variability can drive the evolution of morphological plasticity. With this experiment I had three objectives: (1) to quantify the time scale of morphological change in order to determine if it is compatible with that of natural diet variability, (2) to explicitly examine the adaptive significance of diet-induced morphological plasticity by measuring its effect on foraging efficiency, and (3) to examine the effect of short-term learning (behavioural plasticity) on foraging efficiency and to compare its importance to that of morphological plasticity. Results of this experiment were intended to supplement those of the first experiment and increase our understanding of the factors influencing the evolution of morphological plasticity in sticklebacks. In addition, this experiment allowed me to assess the importance of different types of phenotypic plasticity with regard to foraging efficiency. The second experiment focused solely on the species that exhibited the greatest diet-induced morphological change in the first experiment (the limnetic).

CHAPTER ONE

A COMPARISON OF DIET-INDUCED MORPHOLOGICAL PLASTICITY BETWEEN TWO SPECIES OF THREESPINE STICKLEBACK (GASTEROSTEUS SPP.)

INTRODUCTION

Phenotypic plasticity is environmentally-induced phenotypic change that occurs within an organism's lifetime (Bradshaw 1965; Stearns 1989). A resurgence of empirical and theoretical interest in this phenomenon has brought about a re-evaluation of its ecological and evolutionary significance (West-Eberhard 1989). At one time phenotypic plasticity was thought to result from developmental accidents (West-Eberhard 1989), but new evidence suggests that much environmentally-induced phenotypic variation may be selectively advantageous (Stearns 1983; Bernays 1986; Greene 1989; Spitze 1992; Thompson 1992). This has led some to view plasticity as a trait, subject to evolutionary pressures just as any other phenotypic character (Schlicting and Levin 1986; Scheiner 1993b). The discovery of widespread genotype x environment interaction in natural populations (genetic variation for phenotypic plasticity) further suggests that phenotypic plasticity is an evolutionarily labile character.

Here, I present an empirical study that addresses four fundamental questions concerning the ecological and evolutionary significance of phenotypic plasticity. These are

(1) do my study organisms exhibit plasticity in an adaptive direction; (2) how much of the 'match' between the morphology of a species and its environment is a result of plasticity and, in particular, how much of the morphological difference between species inhabiting different environments is a result of plasticity; (3) have different selective regimes resulted in the evolution of different degrees of plasticity; and (4) is genetic variation for phenotypic plasticity present in contemporary populations? While a variety of studies have addressed (1) and (4) (Lindsey 1962; Bernays 1986; Thompson 1992), few have determined the degree to which species differences in morphology are the result of plasticity (i.e. (2)). Additionally, few studies have compared the degree of plasticity across species for which selection on plasticity is likely to differ (but see Wimberger 1991,1992).

I studied two sympatric species of threespine stickleback (Gasterosteus spp.) from Paxton Lake, British Columbia, Canada (McPhail 1992; Schluter and McPhail 1992). These species are not formally described but are referred to as the 'benthic' species and the 'limnetic' species after the regions of the lake in which they usually forage (University of British Columbia Fish Museum Catalogue #83-351). The benthic species is specialized for littoral foraging and possesses a suite of traits suited to this. Individuals are deep-bodied, possess a small number of short gill rakers and have a wide and terminal gape (fig. 1). The limnetic species is more slender-bodied, with numerous, long gill rakers and a narrow, upturned gape (fig. 1). It is more planktivorous but it also exhibits a seasonal shift in habitat use. Individuals forage in the littoral zone in spring during the breeding season, and then switch to foraging in the water column in summer and fall (Schluter and McPhail 1992; Schluter 1993). Morphological differences between species are largely heritable

(McPhail 1992; Schluter unpubl. data) and strongly affect feeding efficiency and growth rates in different habitats (Bentzen and McPhail 1984; Lavin and McPhail 1986; Schluter 1993, Schluter, unpubl. data).

The benthic and limnetic species are extremely closely related (Nei's genetic distance 0.018; McPhail 1992) and they are probably both descended from the marine threespine stickleback which colonized the lake on two separate occasions (Schluter and McPhail 1992). Comparative evidence suggests that their present morphological and habitat differences are the result of competition-induced character displacement but at present the exact sequence of morphological stages that occurred during their evolution is not resolved. Schluter and McPhail (1992) provide the evidence for character displacement and a discussion of the two main competing phylogenetic hypotheses. The post-glacial history of this region of British Columbia indicates that these two species have coexisted for no more than 13,000 years (Mathews et al. 1970; Clague et al. 1982; Clague 1983). Thus, these species are exceptionally well suited for study because much of their histories is shared, their differences have evolved extremely recently, and this evolution has likely taken place under ecological conditions that are still experienced by the two species.

I addressed the four questions mentioned above in the following way. First, I examined whether plasticity of trophic morphology is adaptive by reversing the 'natural' diets of the two species, and asking whether they become morphologically more similar. I conclude that phenotypic plasticity is adaptive for either or both species if the morphological distinction between them is reduced when they are diet-reversed (fig 1).

Such inference is reasonable because of the effect of trophic morphology on efficiency of prey capture in the two habitats (Bentzen and McPhail 1984; Lavin and McPhail 1986; Schluter 1993). Thus, an individual whose morphology grew to resemble that of the other species when raised on a diet that is characteristic of the other species would likely enjoy an increase in foraging efficiency. An explicit test of the adaptive significance of this morphological plasticity is presented in chapter two.

Second, by comparing morphological differences of the species when diet-reversed with the differences when fed their natural diets, I was able to determine how much of the natural morphological difference between these two species is a direct result of diet. There has been considerable debate over how much of the morphological variation present among some populations is a result of plasticity, especially in systems in which large scale adaptive radiation has occurred (Witte 1984; Meyer 1987; Wimberger 1991,1992). The example most frequently cited is probably that of the cichlid radiation in the African rift lakes but other impressive examples exist (Skulason et al. 1989; Snorrason et al. 1989). Significant adaptive radiation of the Gasterosteus species complex has occurred through colonization of freshwater. The freshwater species have adapted to a variety of conditions present throughout the northern hemisphere (Hagen and McPhail 1970; Bell 1976; Lavin and McPhail 1985; McPhail 1993). My study lends insight into the possible importance of morphological plasticity in these instances.

Third, I compared the degree of plasticity between the two species, one of which is specialized for feeding in the littoral zone (the benthic), and the other of which exploits both habitats seasonally (the limnetic). The limnetic species exploits the littoral habitat in

the spring during reproduction (April - June). Limnetic males build and defend nests in the littoral habitat, and gravid limnetic females use the habitat when searching for prospective mates. Stomach samples indicate that littoral and planktonic prey items are nearly equally represented in the diet of limnetic fish during this life history stage (Schluter and McPhail 1992; Schluter 1993). Once reproduction has ceased, surviving limnetic fish move back into the water column where they feed on zooplankton during the summer and fall (Schluter 1993). In contrast, fish of the benthic species forage in the littoral habitat year round.

The conventional wisdom is that plastic trophic morphology would be beneficial if organisms are faced with significant variability of resource use on the appropriate temporal scale (Gomulkiewicz and Kirkpatrick 1992). Wimberger (1991,1992) compared two congeneric species of cichlids (Geophagus brasiliensis and G. steindachneri) and predicted that differences of diet variability would select for different degrees of morphological plasticity. His results did not bear out this prediction of an interspecific difference. My study addresses the same issue by using a similar type of interspecific comparison. Because individuals of the limnetic species experience significantly greater variability of resource use over the course of their lifetime than do individuals of the benthic species, I expected that limnetics would be more plastic than benthics.

Finally, I employed a full-sib design in my experiment in order to estimate quantitative genetic parameters of phenotypic plasticity. Theoretical results demonstrate that optimal levels of phenotypic plasticity can evolve given the appropriate type of genotype x environment interaction (Via 1987). While some studies were able to quantify

additive genetic variance for phenotypic plasticity (Via 1984a), my design only allowed the characterization of family x diet interaction which is a measure of broad sense heritability of phenotypic plasticity. This parameter is still useful however, because it reveals whether genetic variation for phenotypic plasticity exists. Such a crude partitioning of the genetic variance is usually the norm when dealing with organisms that require extensive laboratory facilities for rearing.

MATERIALS AND METHODS

Crossing Technique and Rearing Program

Fish of both species were raised from artificially fertilized eggs. Eggs from gravid females were stripped into a petri dish by applying gentle pressure to the abdomen in an anterior to posterior direction. Males were sacrificed in MS-222, rinsed, and their testes were removed and minced with forceps in a sterile aqueous saline solution (15% salt by volume). This solution was poured over the egg mass and then left for 3-5 minutes until fertilization had taken place. The egg mass was then rinsed and 30 fertilized eggs were selected randomly. This procedure was repeated to yield 12 full-sib broods of 30 eggs from both the benthic and limnetic species.

Each 30-egg brood was split into a pair of 15-egg half-broods, one being assigned to a live brine shrimp diet (Artemia salina), and the other being assigned to a diet of live blackworms (Tubifex) and frozen bloodworms (Diptera spp.). These two diet treatments were representative of the planktonic and littoral habitats respectively. Consequently, my

experiment allowed a comparison of the two species when raised on their 'natural' diets with the two species when their diets were reversed.

Fertilized eggs were placed in plastic cups with mesh bottoms that were suspended in an aquarium of continuously aerated water. After approximately seven days the eggs hatched and all 15 fish of each half-brood were released into one side of a partitioned 102 L aquarium. There were a total of 24 partitioned aquaria and each aquarium contained a half-brood of both species. Thus the half-broods of each family were raised in different and randomly determined aquaria. Diet treatment was assigned randomly to each aquarium with all aquaria having the same diet treatment on each side of the partition.

Not all fertilizations were performed at the same time due to the sporadic availability of adult fish. The time span between the first fertilization and the last fertilization was approximately one month. The experiment was initiated in the spring of 1992 and terminated in the fall of 1992.

During the first month of life, fish in the littoral treatment were too small to be fed a diet of blackworms. Consequently, all fish were fed a diet of brine shrimp nauplii during this period. After the first month had elapsed, the two experimental diet treatments were used. Fish assigned to the littoral treatment were fed chopped frozen bloodworms for an additional three weeks and then were fed live blackworms for the remainder of the experiment. All fish were fed to satiation on their assigned diet treatment each day. Blackworms (the littoral prey) were administered by depositing the worms into a sand filled petri dish at the bottom of the aquaria. Brine shrimp (the planktonic prey) were released into the water column. These two methods of prey deployment mimic the natural

feeding habitats of the two species. Brine shrimp were cultured in the laboratory and blackworms were purchased weekly from a local pet store. Photo-period was held at a constant 16 L:8 D cycle and temperature was maintained between 17C and 20C.

Measurements

The experiment was terminated in November 1992 when fish had been fed the different diet treatments for approximately 4 months. By this time both species had reached a mean size of approximately 40 mm. This is the adult body size of the limnetic species but the benthic species typically attains sexual maturity at 50mm or larger. All fish were sacrificed in MS-222 and fixed in a solution of 10% formalin for one week. After fixation, fish were stained in a solution of Alizarin Red and 10% KOH in order to render calcified tissue more visible. The fish were then permanently stored in a solution of 37% isopropyl alcohol.

Due to mortality, not all half-aquaria contained the same number of fish when the experiment was terminated. This could potentially confound the comparison between diet treatments if mortality was non-random with respect to diet (Lindsey and Harrington 1972). Unfortunately a comparison between the morphology of surviving and dying fish was not possible because most of the mortality occurred within a few weeks of hatching. Therefore, to rule out natural selection as a cause of morphological differences between groups, I performed a two-way ANOVA with diet and species as factors and mortality level as the dependent variable. Neither factor alone nor their interaction was significant (diet, $0.2 < P < 0.3$; species, $0.05 < P < 0.1$; interaction, $0.7 < P < 0.8$) and there were no

obvious trends in the data to suggest that mortality was biased with respect to morphology. Consequently I concluded that mortality was random with respect to the experimental treatments.

To balance the design for analysis, three fish from each half-aquarium were selected randomly for measurement. This yielded a total of 6 fish per family per species (69 limnetic fish and 70 benthic fish). The number of fish of each species was slightly lower than 72 because five half-aquaria had only 2 surviving fish.

Six characters were measured on each fish: (1) standard length, (2) gape width, (3) gill raker length, (4) gill raker number, (5) head depth, and (6) snout length. Traits (2) through (6) were chosen a priori since they are correlates of foraging efficiency (Bentzen and McPhail 1984; Lavin and McPhail 1986; Schluter 1993). Standard length was used as an overall size measure of each fish. These six traits are also among the most variable between sympatric benthic and limnetic species as well as among allopatric populations of threespine sticklebacks (Hagen and Gilbertson 1972; Gross and Anderson 1984; McPhail 1993; Schluter and McPhail 1992).

All dimensions were measured using an ocular micrometer on a Wild M3C dissecting microscope, except standard length, which was measured using Vernier calipers.

Analysis

All traits except gill raker number were correlated with body size, and consequently a covariate was needed to examine diet-induced morphological changes. To

simplify interpretation, I used standard length as a covariate rather than a composite variable such as a pooled first principal component (PC1). The results were unchanged, however, when PC1 was used instead. Additionally, I use untransformed data in all analyses because this resulted in homogeneous variances between species.

I examined all traits for size x diet interaction by using analysis of covariance. No interaction was evident in either species, implying that the effect of diet was independent of an individual's size (limnetics, $0.05 < P < 0.6$ for all traits; benthics, $0.1 < P < 0.9$ for all traits). Consequently I size-adjusted all traits by least squares regression against standard length using a common slope between treatments for each species. These size-adjusted variables are used in all subsequent analyses.

MULTIPLE SIGNIFICANCE TESTS- Because five traits were examined for phenotypic plasticity, and a variety of comparisons were carried out using these traits, there was a danger that Type I error rates would escalate. I attempted to minimize the number of statistical tests by first carrying out a multivariate test of a given hypothesis using all five traits simultaneously. If the result was statistically significant ($P < 0.05$), I then attempted to decompose the multivariate result into univariate measures to determine the relative role of each trait in the significant multivariate result. There is no single established procedure for performing such a test and Wilkinson (1975) suggests four alternatives. I use univariate tests and employ a sequential Bonferroni procedure (at $\alpha = 0.05$) to guard against Type-I error (Rice 1989). It should be noted however, that it is primarily the relative magnitude of the P-values of the traits that are of interest in such an analysis rather

than their absolute values because the multivariate test establishes an a priori table-wide significance level of $\alpha = 0.05$.

EFFECT OF DIET- If diet-induced phenotypic plasticity is adaptive, then I expected that the benthic and limnetic species would become more similar to one another when their diets were reversed (y2 < y1 in fig 1). Testing this is equivalent to testing whether the sum of effects 'L' and 'B' in figure 1 is greater than zero. An individual fish does not represent the experimental unit in the breeding design because full sibs are not statistically independent. Consequently, I calculated a mean diet-induced change for each of the twelve families of both species for each of the five traits.

Let $\mathbf{B}$ and $\mathbf{L}$ represent two matrices where b_{ij} (or l_{ij}) is the mean diet-induced difference for the ith family and the jth trait of the benthic and limnetic species respectively. I denote the five dimensional (co)variance matrices for $\mathbf{B}$ and $\mathbf{L}$ by $\mathbf{S_B}$ and $\mathbf{S_L}$, and the five dimensional vectors of mean diet-induced differences among family means by $\bar{\mathbf{b}}$ and $\bar{\mathbf{l}}$. I let the vector $\mathbf{t} = \bar{\mathbf{b}} + \bar{\mathbf{l}}$ whose (co)variance matrix is then $\mathbf{S_t} = \mathbf{S_B} + \mathbf{S_L}$ and then tested whether $\mathbf{t} > \mathbf{0}$ using Hotelling's T^2 statistic $T^2 = n(\mathbf{t}-\mathbf{0})^{\mathrm{T}}\mathbf{S_t}^{-1}(\mathbf{t}-\mathbf{0})$ where $\mathbf{0}$ is the five dimensional zero vector and where n=12 is the sample size. This statistic is distributed as $[(n-1)p/(n-p)]F_{p,n-p}$ where p is the number of dependent variables, and $F_{p,n-p}$ denotes the F-distribution with p and $n-p$ degrees of freedom (Johnson and Wichern 1982).

The above multivariate analysis revealed whether the overall 'morphological gap' between the two species was narrowed. Subsequently, I carried out univariate t-tests to

determine if the sum of 'L' and 'B' in figure 1 was greater than zero using the sequential Bonferroni procedure mentioned above. This revealed the nature of the multivariate difference.

INTERSPECIFIC COMPARISON.-The above analysis does not reveal whether diet-induced changes were exhibited equally by both species or if one species was primarily responsible for the 'narrowing of the gap' when both were diet-reversed. My expectation was that the gap would be narrowed primarily by diet-induced change in the limnetic species (i.e. L > B in fig 1), thus reflecting greater adaptive phenotypic plasticity in the species with the more variable natural diet.

To test this expectation I performed a one-way multivariate analysis of variance (Johnson and Wichern 1982) comparing the mean diet-induced change of the twelve benthic families with the mean diet-induced change of the twelve limnetic families for all five traits. A significant Wilk's lambda indicates that there is a significant overall difference between species in phenotypic plasticity. The multivariate result was then decomposed using a sequential Bonferroni procedure (Rice 1989) on univariate ANOVA results for each trait.

FAMILY x DIET INTERACTION-For phenotypic plasticity to evolve, genetic variation for plasticity must exist in the population (Via 1987). My experimental design allowed me to estimate family x diet interaction. This is a measure of broad sense genetic variation for phenotypic plasticity and thus reflects whether plasticity has a genetic component. I

estimated the significance of this interaction using a two-way mixed model, multivariate analysis of variance (Johnson and Wichern 1982). Separate MANOVAs were carried out for each species. Family was considered as a random factor in this analysis and diet was considered as a fixed factor.

A potential complication in calculating family x diet interaction arises from the confounding effects of micro-environment. Each of the 15 fish from a half-brood that were raised on the same diet, were also raised in the same half-aquarium, and thus they are not strictly independent. All of these fish experienced the same micro-environmental (aquarium) effects during their growth. Because I assumed that sibs are independent when calculating the family x diet interaction term, my findings must be regarded as tentative. A previous quantitative genetic analysis of sticklebacks by Lavin and McPhail (1987) demonstrated, however, that aquarium effects on size-corrected measurements are negligible, lending support to my assumption.

There is considerable difficulty in interpreting the relationship between the dependent variables and the factors in a two-way MANOVA when there is a significant interaction effect (Morrison 1976; Johnson and Wichern 1982). My purpose with this test was mainly to determine whether there was significant overall genetic variation for plasticity and consequently I did not attempt to examine the effect of diet or family in each species using this procedure. To probe the nature of the genetic variation for plasticity I calculated two-way univariate ANOVA's for each trait of both species.

All analyses were carried out using Systat 5.01 on an IBM-compatible microcomputer (Wilkinson et al. 1992).

RESULTS

Effect of Diet

All diet-induced changes were in a direction that is suggestive of adaptive phenotypic plasticity. Limnetic fish raised on a littoral diet developed a morphology that was displaced toward that of the benthic species relative to control fish raised on a planktonic diet. Benthic fish raised on a planktonic diet developed a morphology that was displaced toward that of the limnetic species relative to control fish raised on a littoral diet. This trend was exhibited by all morphological characters (fig. 2) except gill raker number which displayed no trend of change.

The results of the multivariate analysis reveal that the 'morphological gap' between the benthic and limnetic species was significantly reduced by diet-reversal (Hotelling's $\mathbf{T}^2$ = 45.14, df = 5 and 7, $0.01 < P < 0.05$). Thus, when considering all traits simultaneously, diet affects the degree to which these species differ morphologically. This suggests that differences in diet may contribute to the morphological difference between the two species in the wild.

Table 1 presents the percent reduction of the morphological gap between the two species caused by diet-reversal for each of the five traits. Percent reductions ranged from -1 percent for gill raker number to 58 percent for head depth. Also presented in table 1 are the results of the univariate t-tests for each trait. Gill raker length was the only trait that was significant using the sequential Bonferroni procedure. It is informative, however, that all traits except gill raker number exhibited a diet-induced change in the same direction.

Additionally, head depth had a P-value less than 0.05 which suggests that it also contributed to the multivariate significance.

Interspecific Comparison

Figure 2 and table 2 suggest that the limnetic species tends to be more plastic than the benthic species in all traits except snout length and gill raker number (which showed virtually no plasticity). MANOVA confirmed that the two species differed in their amount of plasticity, although statistical significance is marginal ($P = 0.05$). Univariate ANOVAs (table 2) revealed that only gill raker length displayed a significant difference in the magnitude of plasticity between the two species. All other traits had P-values at least an order of magnitude larger than gill raker length. Thus, the degree of plasticity of the two species appears to be more distinct in some traits than in others, suggesting that the benthic and the limnetic species differ not only in the magnitude of plasticity but in the pattern of plasticity among these morphological characters as well.

Family x Diet Interaction

Multivariate analysis revealed the presence of significant family x diet interaction in both species ($P < 0.001$) indicating that there were differences among families in the extent and/or direction of diet-induced change. This is a measure of broad sense heritability of phenotypic plasticity. Thus, there is genetic variability for morphological plasticity present in both species' populations.

Results of the univariate analysis are presented in table 3. Family x diet interaction in the benthic species is exhibited primarily by gape width, although gill raker length and snout length are nearly significant. Family x diet interaction in the limnetic species is exhibited primarily by gape width. This suggests that morphological plasticity has evolutionary potential and could respond to natural selection in both species of sticklebacks .

DISCUSSION

The ecology of morphological plasticity is not well understood and is just beginning to receive empirical attention (Meyer 1990; Witte et al. 1990; Wimberger 1991,1992). The ecological conditions that select for morphological plasticity are still unclear. Yet this knowledge is necessary to understand the interplay between selective regimes, plasticity, and morphological evolution. The wider effects of morphological plasticity on niche partitioning, speciation and adaptive radiation are also poorly understood. The extent to which plasticity accounts for morphological variation within and between species would lend some insight to this problem.

This study addressed four important issues of morphological plasticity using two sympatric species of stickleback. First, I demonstrated that when the two species are diet-reversed, their morphologies become more similar overall. This suggests that either or both species exhibits adaptive morphological plasticity. The individual traits that contributed most to this overall morphological change were gill raker length and perhaps

head depth. Both of these traits are probably important with respect to feeding efficiency (Bentzen and McPhail 1984; Lavin and McPhail 1986; Schluter 1993).

The second issue I addressed was how much of the interspecific difference in morphology of these species can be attributed to plasticity. The percentage of the 'morphological gap' between the two species that was closed by diet-reversal ranged from -1 percent for gill raker number to 58 percent for head depth. Thus a considerable amount of the morphological difference between the two species in the wild might be attributable to diet-induced morphological change.

Third, I compared degree of plasticity between the two species, on the expectation that greater natural diet-variability in the limnetic species would drive the evolution of greater plasticity in that species. The two species did exhibit a significant difference in overall level of phenotypic plasticity and this difference was primarily attributable to a greater plasticity of gill raker length in the limnetic than in the benthic species. Gill raker length has been strongly implicated as a determinant of foraging efficiency in sticklebacks (Bentzen and McPhail 1984; Lavin and McPhail 1986; Schluter 1993) and therefore these results lend support to the hypothesis that diet variability can select for morphological plasticity. Head depth and gape width also tended to be more plastic in the limnetic than in the benthic species but these differences were not statistically significant (fig 2).

Lastly, I addressed whether contemporary populations of benthics and limnetics maintain genetic variation for morphological plasticity. Theoretical work suggests that optimal levels of phenotypic plasticity can evolve given appropriate genotype x environment interaction (Via and Lande 1985; Via 1987). The family x diet interaction I

demonstrate reveals a broad sense heritability of plasticity (Via 1984a, 1984b). This heritability includes variation due to dominance and epistatic interactions as well as additive genetic variance (Falconer 1989). While this does not completely satisfy the conditions required for the evolution of phenotypic plasticity, it does reveal that plasticity is genetically determined.

When making comparisons between species it is often difficult to distinguish the effect of phylogeny from the effect of recent natural selection (Lauder 1982). Because the benthic and limnetic species have both recently evolved from a common marine ancestor (McPhail 1993), and because both are relatively 'young' species, I can be reasonably confident that phylogenetic effects are not responsible for their differences in plasticity.

Additionally, the wealth of natural history information of these two species of stickleback and the well documented glacial history of this region, suggest that the environmental context in which these two species have evolved is relatively well-understood. Consequently, the adaptive nature of all diet-induced morphological changes, the greater degree of plasticity in the limnetic species, and the presence of genetic variation for plasticity, together provide compelling evidence to support the hypothesis that morphological plasticity has evolved as a result of diet variability. It has often been suggested that diet variability might drive the evolution of trophic morphological plasticity but there has been little such evidence.

At this point however, two alternative hypotheses for the observed results deserve mention. One involves the possibility of differences between the two species in their ability to ingest prey. Because benthic fish are generally larger than limnetic fish in most

dimensions, it may be easier for a benthic fish to ingest a planktonic prey than for a limnetic fish to ingest a littoral prey. Assuming that diet-induced morphological change results from mechanical stress (as discussed below), limnetic fish would likely exhibit greater morphological plasticity than benthic fish. However, if this were the case, one would also expect there to be an overall effect of body size on the degree of plasticity. No interaction between size and the effect of diet was evident in the data and consequently I do not believe this to be the explanation for my results.

The second alternative basis for the interspecific difference in plasticity may lie in differences in the way benthic fish and limnetic fish are constructed. Since their divergence from a common ancestor, the limnetic species has evolved a less robust morphology than the benthic species. For example, gill rakers of the limnetic species are not only longer and more numerous than those of the benthic species, but they are more slender as well. Thus, if slender-built morphology is more susceptible to stress-induced change, this would explain how the difference in plasticity between the two species is determined. Conceivably, such differences in robustness have evolved merely as an incidental byproduct of evolutionary divergence in body form, in which case interspecific differences in plasticity are simply a (non adaptive) correlated response to selection on the mean value of each trait. If, however, robustness of morphology can evolve independently of the mean values of morphological traits, then such structural differences may be the proximate mechanism by which adaptive evolution of plasticity is realized.

Diet-induced morphological plasticity could result from either nutritional differences between diets or from differences in the mechanics of prey ingestion.

Nutritional effects are confounded with mechanical effects in my experiment. Nutrition can be an important determinant of fish morphology (Halver 1984; Wimberger 1993). I feel, however, that differences in the mechanics of prey ingestion are more important in this study. If nutritional differences were important, diet-induced morphological changes would likely have exhibited a more random pattern (Wimberger 1993). The pattern of diet induced change demonstrated is consistent with the difference in morphology observed between many littoral foraging and plankton foraging species (Lavin and McPhail 1985, 1986; Schluter and McPhail 1993). Littoral and planktonic ecomorphs likely result from the mechanical requirements of foraging in these habitats rather than from nutritional effects.

I suspect that mechanical stress is the cause of observed morphological plasticity in the traits I examined, because these structures are composed of either cartilage or bone. It has long been realized that structures made of bone remodel and change shape depending on the stresses imposed upon them (Lanyon 1984; Lanyon and Rubin 1985). The lack of diet-induced change in gill raker number is consistent with this hypothesis, because whereas mechanical stress can change the shape of particular structures it can not easily alter their number.

Behavioural plasticity also likely plays an important role in adaptation to resource variability. Changes in foraging behaviour have large effects on foraging efficiency (Dill 1983; Ehlinger 1989a, 1989b) and behaviour is probably amenable to more rapid change than morphology. For example, very different modes of foraging are used to exploit plankton and benthos, and individual fish switch rapidly between them when moving

between habitats (Schluter 1993). An experiment which tests the adaptive significance of behavioural plasticity versus morphological plasticity is presented in chapter two.

Evolutionary Implications

Whether phenotypic plasticity retards or enhances evolution is still a matter of some debate (West-Eberhard 1989; Stearns 1989). The distinction may be particularly important when considering the Gasterosteus species complex. Invasion of freshwater by marine G. aculeatus is pervasive throughout the holarctic region (Wootton 1976). Colonization of freshwater occurred as the Pleistocene glaciation ended and dramatic adaptive radiation ensued (Hagen and McPhail 1970; Bell 1976; McPhail 1993). Even slight differences among bodies of freshwater in the same drainage basin have led to fine scale adaptation in resident sticklebacks (Lavin and McPhail 1985). Trophic morphology maps remarkably well onto lake ecology in all populations examined (Lavin and McPhail 1985; Schluter and McPhail 1992). Heritability of trophic morphology also provides compelling evidence that this diversity is the result of evolutionary change.

What has not been known is the extent to which this radiation owes its diversity to phenotypic plasticity. A comparison of several populations of sticklebacks in British Columbia (Schluter and McPhail 1992) has shown that gill raker length, gape width and gill raker number are among the most variable traits among stickleback populations. Given that gill raker length has exhibited considerable phenotypic plasticity, it is possible that some of this interpopulation variability is environmentally-induced.

The effects of phenotypic plasticity on adaptive radiation and speciation in Gasterosteus is not clear. It is evident that not all sticklebacks are equally phenotypically plastic, but the extent to which plasticity plays a role in the evolution of species pairs such as the benthic and the limnetic is unknown. Given that trophic character displacement is an important component of evolution and speciation in sticklebacks (Schluter and McPhail 1992), it is possible that plasticity of trophic traits plays a very important role as well.

Table 1. Absolute magnitude of the 'morphological gap' between species when raised on their 'natural' diets and when diet-reversed. Values are calculated using the mean of the twelve family means from each species for each trait. Units are in millimeters except for gill raker number. Asterisks indicate the results of univariate t-tests for a reduction of the morphological gap between species.

Trait	Natural Diet	Diet-Reversed	Percent Reduction
Gape Width	0.585	0.339	42
Gill Raker Length	0.456	0.283	38***
Gill Raker Number	5.291	5.319	-1
Head Depth	0.477	0.201	58*
Snout Length	0.665	0.469	29

*** $P < 0.001$ *$0.01 < P < 0.05$

Table 2. Percentage difference in each morphological character under diet-reversal. Each value was calculated by dividing the absolute value of mean diet-induced change by the mean value of that character when the species was raised on its 'natural' diet. Asterisks indicate a significant difference between species as tested using univariate ANOVAs.

Character	Benthics (% difference)	Limnetics (% difference)
Gape Width	1.8	7.1
Gill Raker Length**	5.1	11.0
Gill Raker Number	1.4	1.0
Head Depth	0.8	2.5
Snout Length	2.6	2.8

** $P < 0.01$

Table 3. F-ratios from univariate ANOVAs for family x diet interaction (F x D df =11, Error df = 46 for all values).

Trait	Species: Benthic	Species: Limnetic
Gape Width	7.34***	13.76***
Gill Raker Length	2.29*	0.68
Gill Raker Number	1.58	1.99
Head Depth	1.55	1.44
Snout Length	2.18*	1.52

*** P < 0.001 * 0.01 < P < 0.05

Fig 1. The benthic species (left) and the limnetic species (right). Also the expected pattern of diet-induced morphological change. The four points represent the mean value of a trait for the four species x diet combinations. The diet-induced change of the limnetic species (L) is predicted to be greater than the diet-induced change of the benthic species (B) if diet-variability has driven the evolution of morphological plasticity. The difference between the two species when raised on their 'natural' diets (y1) is expected to be greater than the difference between the two species when they are diet-reversed (y2) if morphological plasticity is adaptive.

Fig 2. Plots of the four species x diet combinations for all traits except gill raker number (which exhibited no plasticity). Plots are the mean of the 12 family means in each combination (i.e. N=12 for each of the means for each trait) and the 95 percent confidence intervals. Units are in millimeters.

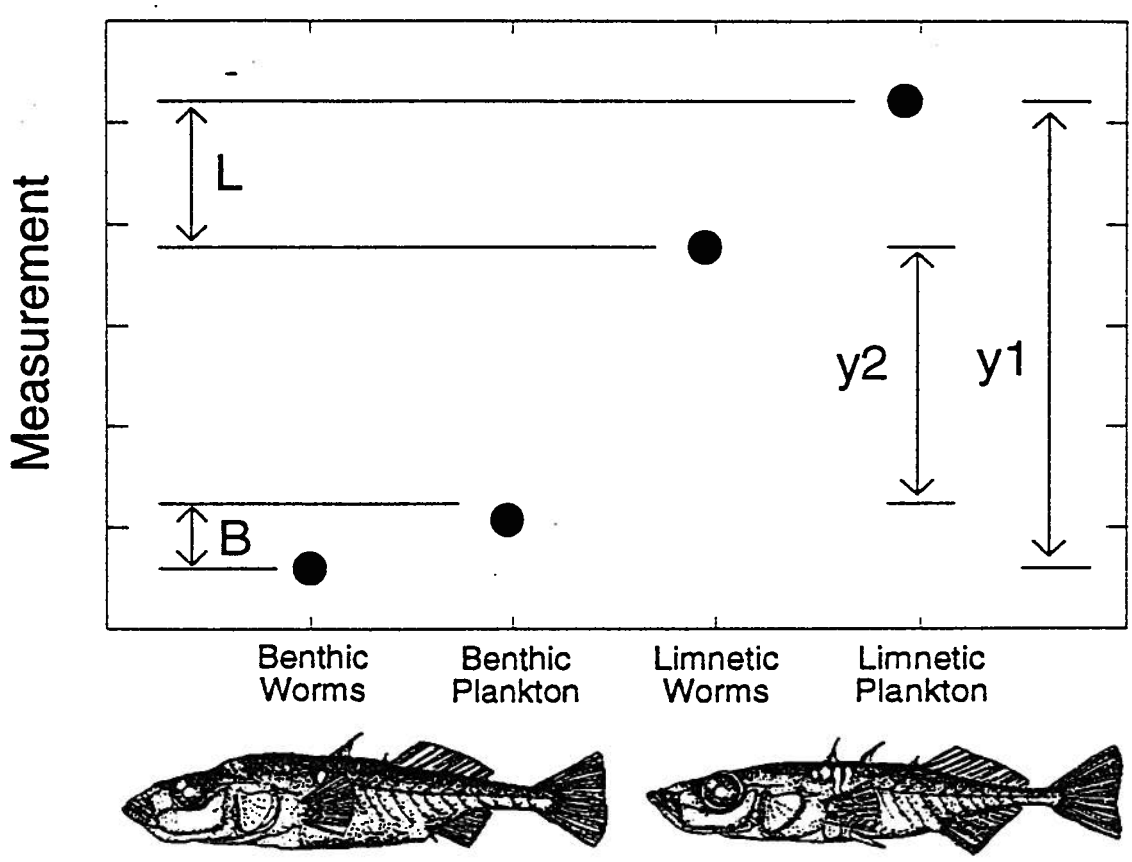

Measurement
L
B
y2
y1
Benthic Worms
Benthic Plankton
Limnetic Worms
Limnetic Plankton

Figure 1

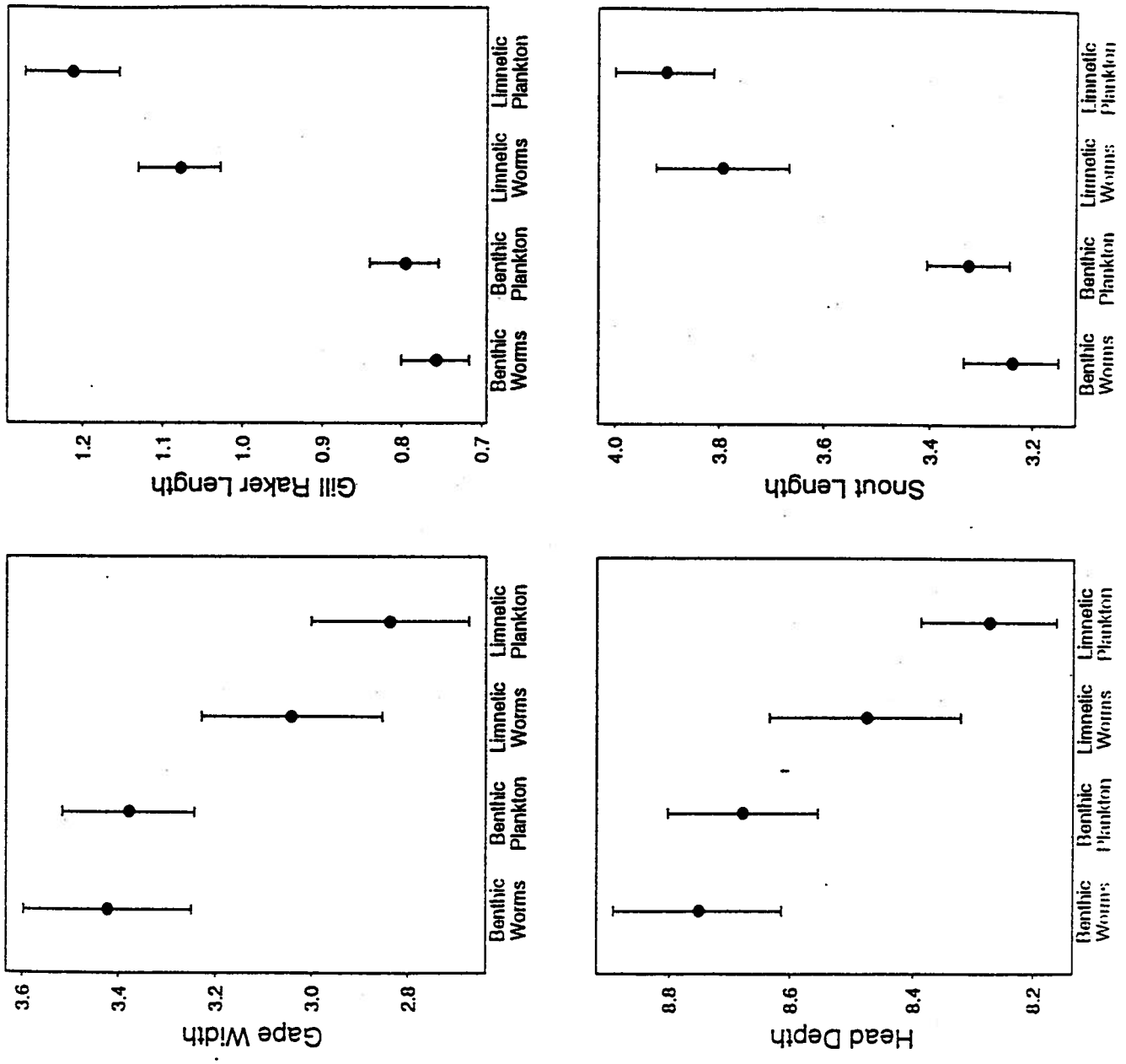

Gape Width
3.6
3.4
3.2
3.0
2.8
Benthic Worms
Benthic Plankton
Limnetic Worms
Limnetic Plankton
Gill Raker Length
1.2
1.1
1.0
0.9
0.8
0.7
Benthic Worms
Benthic Plankton
Limnetic Worms
Limnetic Plankton
Head Depth
8.8
8.6
8.4
8.2
Benthic Worms
Benthic Plankton
Limnetic Worms
Limnetic Plankton
Snout Length
4.0
3.8
3.6
3.4
3.2
Benthic Worms
Benthic Plankton
Limnetic Worms
Limnetic Plankton

Figure 2

CHAPTER TWO

THE ECOLOGICAL SIGNIFICANCE OF MORPHOLOGICAL AND BEHAVIOURAL PLASTICITY

INTRODUCTION

Recently, interest in the evolution of phenotypic plasticity has increased (Stearns 1989; West-Eberhard 1989; Scheiner 1993a) and, although theoretical research has advanced significantly (Levins 1968; Via and Lande 1985; Gomulkiewicz and Kirkpatrick 1992; Leon 1993; Gavrilets and Scheiner 1993a, 1993b), empirical research, especially on natural populations, has lagged behind. While there have been several examinations of phenotypic plasticity in natural populations (Lindsey 1962, 1972; Schlicting and Levin 1986; Dodson 1989; van Noordwijk 1989; Witte et al. 1990; Wimberger 1991, 1992), the majority of empirical tests of theory on the evolution of phenotypic plasticity have come from laboratory studies of Drosophila (Scheiner 1993a). These studies contribute substantially to our understanding of how phenotypic plasticity evolves but, ultimately, we want to know if theoretical predictions are borne out in natural populations.

In this chapter I extend the results of chapter one that suggested diet variability can drive the evolution of plastic trophic morphology. In chapter one I demonstrated an association between diet variability and the degree of diet-induced morphological plasticity in two sympatric species of threespine stickleback. Additionally, I documented the heritability of morphological plasticity and provided evidence that suggests diet-induced

morphological change is adaptive. The present chapter extends these results in three ways. First, I examine the time scale over which diet-induced morphological change occurs to determine if it is compatible with the time scale of natural diet variability. Second, I examine the adaptive significance of diet-induced morphological plasticity by measuring its effect on foraging efficiency. Third, I examine the effect of short-term learning (behavioural plasticity) on foraging efficiency and compare its importance to that of morphological plasticity.

Theoretical work suggests that the degree of plasticity that evolves depends upon the rate of occurrence of the plastic response to environmental cues (Gomulkiewicz and Kirkpatrick 1992; Leon 1993). Traits are usually classified as labile or non-labile in such theoretical analyses. Labile traits are those whose plastic response to environmental cues is effectively immediate, whereas non-labile traits are those whose expression is plastic but whose value is fixed at some point during development. In reality it is possible for traits to be anywhere between these two extremes. While many studies examine morphological plasticity, few determine whether the time scale of the plastic response is compatible with that of the environmental variability for which the plasticity is suspected to be an adaptation.

Morphological and Behavioural Plasticity

Studies of the adaptive significance of diet-induced morphological plasticity are rare, and in those that exist (e.g. Thompson 1992), few consider of the potential effect of diet-induced behavioural change. Yet, behavioural plasticity can have a large impact on

foraging efficiency (Werner et al. 1981; Dill 1983; Ehlinger 1989a, 1989b, 1990; Croy and Hughes 1991). Consequently, where diet-induced morphological change is accompanied by a change in foraging efficiency, the effects of behavioural and morphological plasticity often are confounded.

My experiment is designed explicitly to examine both types of phenotypic plasticity. I define learning (behavioural plasticity) as behavioural changes that take place on a time scale short enough to preclude most morphological change. The effect of learning is measured as the changes in foraging efficiency that accrue from short-term experience with a prey type.

Morphological plasticity is relatively easy to measure but its effect on foraging efficiency is more difficult to quantify. Long-term exposure to a particular prey may alter not only trophic morphology but also neural morphology and thus result in indirect behavioural changes (Krebs 1990; Healy et al. 1994 and references therein). Also, learning (as defined above) may be morphology-dependent. That is, its effects on foraging efficiency may change as morphology changes (an interaction). Consequently, it is not possible to completely disentangle the effects of morphological and behavioural plasticity. Because of this problem I define the effect of morphological plasticity as the usual effect of diet-induced changes in trophic morphology plus any effect of changes in behaviour that result from long-term exposure to prey. This effect is measured by documenting changes in foraging efficiency that take place with long-term exposure to prey.

Plasticity and Sticklebacks

My study organism is the planktivorous stickleback (Gasterosteus sp.) found in six lakes of coastal British Columbia, Canada (McPhail 1993). The species is not formally described but is termed the 'limnetic' species because it forages predominantly on calanoid copepods (Diaptomus spp.) in the 'limnetic' (water column) habitat of lakes. The limnetic species coexists with a 'benthic' species which forages predominantly on invertebrates (gammarids) in the littoral region of the lakes. The two species are morphologically distinct, and differences in morphology have important effects on foraging efficiency and growth in the two habitats (Bentzen and McPhail 1984; Schluter 1993, 1994).The limnetic species has a long snout, long gillrakers, a slender head and a narrow gape relative to the benthic species (McPhail 1992). These morphological differences are largely heritable (McPhail 1992; chapter one) and are thought to have evolved as a result of competition for resources and character displacement (Schluter and McPhail 1992, 1993).

In chapter one I demonstrated that the limnetic species exhibits greater diet-induced morphological plasticity than the benthic species. Because limnetics forage in the benthic habitat during their breeding season (Schluter and McPhail 1992; Schluter 1993), they experience substantial resource variability. In contrast, benthic fish forage in the benthic habitat throughout their lives and therefore have a relatively monotonous diet. Therefore, in chapter one, I suggested that greater diet-induced morphological plasticity in the limnetic species may have evolved to cope with this high degree of resource variability.

In this chapter I document the time scale over which morphology changes by keeping limnetics on a diet of either calanoid copepods (limnetic prey) or gammarids (benthic prey) and sampling fish from these two treatments over time. A comparison of this time scale with that of the natural resource variability arising from the breeding cycle allows me to determine if the two are compatible. Also, by conducting foraging trials on gammarid prey items with fish from the two diet groups, I can quantify the effect of morphological plasticity and learning. If diet-induced morphological plasticity has evolved as a result of diet variability, then limnetics with gammarid-induced morphology should be more efficient foragers on gammarids than limnetics with calanoid-induced morphology (all else being equal).

MATERIALS AND METHODS

All fish were taken from Paxton Lake, British Columbia, Canada in mid-April 1993 using dipnets. Each individual (25mm - 30mm standard length) was randomly assigned to a diet of either calanoid copepods (Diaptomus spp.) or gammarid amphipods (Gammarus lacustris). These diet treatments represent prey commonly found in the natural foraging habitats of the limnetic species (Schluter and McPhail 1992). Fish were kept in eight 102 L aquaria (four per diet treatment) at initial densities of approximately 30 individuals per aquarium. These densities declined throughout the summer as a result of mortality and sampling for foraging trials. All fish that died during the course of the summer were kept for comparison with surviving individuals. This allowed me to check that morphological differences between diet treatments were the result of diet-induced plasticity rather than

differential mortality. Photoperiod was constant (16L:8D cycle) for the entire period and temperature varied with ambient temperature (between 13 and 19 degrees Celsius).

Diet Treatment

I used a diet-switching experimental design (fig. 3). The pre-switch period was longest and was meant to induce an effect of both learning and morphological plasticity on foraging efficiency. The shorter post-switch period was meant to induce only a learning effect. There were three sets of foraging trials, each with a different duration of pre-switch diet exposure (sets 1, 2 and 3 had 18, 39 and 72 days of exposure respectively). The purpose was to estimate the time scale over which morphology changes, and to determine the effect of different degrees of morphological change on foraging efficiency. The post-switch period was ten days for all three sets. Although the duration of the post-switch period is somewhat arbitrary, ten days probably is sufficient to produce efficiency changes through learning (Werner et al. 1981; Ehlinger 1989a; Schluter 1993) yet short enough to preclude most morphological change.

Switching involved selecting a random sample of fish (18, 24 and 24 fish for sets 1, 2 and 3 respectively) from each of the two diet treatments. Half the fish were kept on the same diet, and the remaining half were given a diet opposite to what they had experienced during the pre-switch period. This design provided three sets of four experimental treatments: calanoid pre-switch/calanoid post-switch (C/C), calanoid pre-switch/gammarid post-switch (C/G), gammarid pre-switch/calanoid post-switch (G/C), and gammarid pre-switch/gammarid post-switch (G/G) (fig. 3).

Foraging Trials

These trials were to determine how experience with gammarids or copepods affects foraging efficiency on gammarids. In each trial a single fish was placed in a 102 L aquarium containing ten gammarids (mean length = 3.47 [SD = 0.53], mean width = 1.06 [SD = 0.18]) on a sand substrate. I recorded the time at which each of the following events occurred: orientation towards substrate or prey item, prey attacked on substrate, prey attacked in water column, prey spat out, end of orientation towards substrate or prey item, prey swallowed, 'empty' strike on substrate and 'empty' strike in water column. Empty strikes are instances where the fish appeared to strike at nothing, or at debris in the aquarium other than gammarids. Because foraging efficiency varies with satiation level, trials ended either after ten minutes or after the consumption of two prey items, whichever came first.

Measures of Foraging Efficiency and Morphology

The information recorded during each foraging trial allowed me to calculate four measures of foraging efficiency: two for searching efficiency and two for handling efficiency.

The searching efficiency measures are (1) time from fish introduction to first attack (henceforth termed 'latency time') and (2) search time per prey item. Search time/prey was calculated by dividing the total search time by the number of prey items consumed plus one. One was added to the denominator to prevent division by zero in instances

where a fish searched for, but did not consume, prey items. Both variables were log transformed to normalize their distributions.

The handling efficiency measures are (3) handling time per prey item consumed and (4) number of attacks required per prey item consumed. Both were calculated by dividing total handling time, or number of attacks, by the number of prey consumed plus one. Both measures were log transformed to normalize their distributions, and both were size-corrected by least-squares regression against standard length because they were correlated with body size. This regression was carried out on combined data from the three different pre-switch durations (the three sets).

Five morphological characters were measured on each fish: (1) gillraker length, (2) head depth, (3) gape width, (4) snout length, and (5) standard length. Characters (1) through (4) were chosen because chapter one demonstrated they have a plastic response to diet, and character (5) was used as a covariate for size-correction of various measurements. All were log transformed to equalize their variances. Although other morphological characters probably exhibit diet-induced plasticity, these characters provide an index of overall trophic morphology. Characters (1) through (4) were measured using an ocular micrometer on a Wild M3C microscope at 6.4x - 16x magnification and standard length was measured using Vernier calipers.

Size-corrected morphological characters were used in all analyses except for that of time scale. Size-correction was carried out by least-squares regression of each character against standard length using combined data from all three sets. The size-corrected variables were adjusted to the mean standard length of the combined data.

Analysis

Most of the analyses are one-tailed. The analysis for diet-induced morphological change is one-tailed because chapter one demonstrated a consistent direction to all diet-induced change. Likewise, the analyses for changes in foraging efficiency are one-tailed because morphological and behavioural experience with gammarids is never expected to decrease foraging efficiency on gammarids. Analyses are univariate in all cases where a directional alternative hypothesis is appropriate because it is difficult to incorporate directional hypotheses into multivariate statistical procedures. In these instances, I correct for table-wide significance levels using the sequential Bonferroni technique (Rice 1989). All statistical analyses are performed using Systat 5.02 for Windows (Wilkinson et al. 1992).

To rule out differential mortality (natural selection) as a cause of observed morphological change, I conducted a one-way MANOVA (Johnson and Wichern 1982) on the fish that died. I used the pre-switch diet treatment as the independent variable and the morphological characters that exhibited change as the dependent variables. If differential mortality caused the observed pattern of morphological change, then morphology should differ between diet groups in a direction opposite to the difference in morphology of surviving fish. The effect of pre-switch diet was not significant ($F_{2,80} = 2.32$, $P = 0.11$) and the direction of difference between diet treatments was the same in both dead and surviving fish. This indicates that diet-induced plasticity occurred in both

the fish that died and those that survived. Therefore, the morphological changes are not the result of biased mortality.

TIME SCALE. - An assumption in the experimental design is that most diet-induced morphological change occurs on a time scale longer than ten days. This assumption was tested by combining data from the three pre-switch durations (sets) for treatments C/C and C/G and then comparing the two treatments using a one-way MANOVA on the four morphological variables. The same procedure was conducted with groups G/C and G/G. The results of these tests confirm that ten days of diet treatment is not sufficient to induce a detectable morphological change (C/C vs. C/G, $F_{4,61} = 0.568$, $P = 0.69$; G/C vs. G/G, $F_{4,61} = 0.589$, $P = 0.67$).

To determine the time scale over which morphology does change I compared the morphology of treatments C/C and C/G combined with treatments G/C and G/G combined across the three pre-switch durations (sets). Thus I had two 'morphology' groups from each pre-switch duration (set) to compare (the groups were determined by pre-switch diet treatment, fig. 3). These groups were compared using a univariate one-tailed ANCOVA (Neter and Wasserman 1974) for each morphological character for each pre-switch duration. Standard length was the covariate. The pre-switch duration at which the two groups are significantly different is an estimate of the time necessary to induce morphological change.

EFFECT OF MORPHOLOGICAL PLASTICITY AND LEARNING. - Treatments C/C, C/G and G/G (fig. 3) were used to estimate the effect of morphological plasticity and learning. If diet-induced morphological change affects foraging efficiency, then divergence in morphology between the two pre-switch diet treatments from set 1 to 2 to 3 should be paralleled by divergence in foraging efficiency between groups [C/C + C/G] and [G/G] because these two groups differ in pre-switch diet. Testing for such divergence tests for the effect of morphological plasticity. The effect of learning was tested by comparing group [C/C] with [C/G + G/G] because these two groups differ in post-switch diet.

I used the following regression model to test for both effects simultaneously:

$$Y = \alpha + \beta \bullet duration + \delta_{pre} \bullet \gamma_{pre} \bullet duration^2 + \delta_{post} \bullet \gamma_{post} \ .$$

Y is the measure of foraging efficiency and *duration* is the length of pre-switch diet treatment in days. δ_{pre} and δ_{post} are indicator variables specifying the type of pre- or post-switch diet: δ_{pre} or δ_{post} equals 1 if the diet treatment is calanoids and -1 if the diet treatment is gammarids. This model assumes that foraging efficiency is equal in treatments C/G and G/G ($Y = \alpha$) at zero pre-switch duration and that efficiency of the two pre-switch diet groups ([C/C and C/G] versus [G/G]) diverges non-linearly with time (represented by the term, $\gamma_{pre} \bullet \delta_{pre} \bullet duration^2$); the quadratic was a good first approximation to this non-linear divergence (fig. 3). Because there was no significant interaction between the effect of post-switch diet type and pre-switch duration for any foraging efficiency variable ($F_{1,56}$

= 2.161, 0.106, 0.037, 0.038 for attacks/prey, handling time/prey, search time/prey, latency time), the effect of learning is represented by a constant displacement (γ_{post}) of C/C from C/G for all pre-switch durations. The model also specifies a common linear term for all three treatments ($\beta \bullet duration$). If γ_{pre} or γ_{post} are significantly different from zero then foraging efficiency was affected by morphological plasticity or learning, respectively.

As an additional analysis for the effect of morphological plasticity I conducted a univariate multiple regression for each (un-corrected) efficiency variable using those (size-corrected) morphological variables that exhibited diet-induced change as independent variables. If the characters that exhibited a diet-induced change (or any characters correlated with them) actually do affect foraging efficiency, the efficiency variables should depend on them. This analysis was carried out on data from all three pre-switch durations combined.

RESULTS

Time Scale

Head depth was the only morphological character that exhibited significant diet-induced plasticity, and then only in the longest pre-switch duration (table 4). None of the characters exhibited statistically significant plasticity before this set although the trend in gape width appeared to plateau (or even decline) after the second set (fig. 4). Combining sets 2 and 3 for gape width, however, results in a statistically significant difference between the two diet groups ($F_{1,92} = 4.00$, $P = 0.024$). This suggests that small effect size and/or high variability result in the need for larger sample sizes. There was no consistent

pattern of diet-induced plasticity in gillraker length or snout length across the three pre-switch durations (sets). These results differ from those in chapter one which demonstrate substantial plasticity in all four morphological characters. Fish were kept on different diets from an earlier age in the previous study, however, and this suggests that some traits might exhibit non-labile plasticity. Perhaps, differences in diet and/or growth rate between the present and previous experiments accounts for the difference in plasticity as well.

Effect of Morphological Plasticity

Foraging efficiency on gammarids continually improved as an individual's experience with gammarids increased up to 72 days; all measures of forging efficiency, except latency time, significantly diverged between the two pre-switch diet treatments (i.e. γ_{pre} is significantly different from zero, table 5; C/C [and C/G] diverge from G/G as pre-switch duration increases, figure 5). This increase in foraging efficiency parallels, and is roughly on the same time scale, as diet-induced morphological change (fig. 4). This suggests that part of the change in efficiency may be due to plasticity in the characters examined.

In general, the pattern exhibited by the foraging efficiency variables suggests that divergence in efficiency between treatments C/C (or C/G) and G/G over time is due to both the increased efficiency of treatment G/G and the decreased efficiency of treatment C/C (or C/G). Such an interpretation should be treated cautiously, however, because a trend of decreased efficiency irrespective of treatment group across the three pre-switch durations would produce this same pattern.

Multiple regression analyses of specific morphological characters reveal that only head depth is significantly related to handling time/prey (table 6). Apparently gape width is not related to any of the efficiency variables. Note, however, that the R^2 values are low (handling time/prey, 0.049; attacks/prey, 0.032; search time/prey, 0.016; latency time, 0.007), and this implies large variations in foraging efficiency that are not explained by variation in either morphological character. Although measurement error was undoubtedly partly responsible for these low R^2 values, other additional factors (e.g., unmeasured traits) probably also play important roles in determining foraging efficiency.

Effect of Learning

Both searching efficiency variables were improved by post-switch experience with gammarids (γ_{post} for latency time, $P = 0.02$; search time/prey, $P = 0.007$; table 5) but, using the sequential Bonferroni technique, only search time/prey was significant. This variable displayed both an effect of morphological plasticity and an effect of learning (table 5; fig. 5[iii]). Lack of a significant effect of learning on latency time might be the result of low statistical power since latency time was consistently highest in treatment C/C at each of the three pre-switch durations (fig. 5[iv]). Neither handling efficiency variable exhibited a significant effect of learning nor did they display any consistent pattern at each pre-switch duration.

A comparison of the elevations of the three regression lines at 72 days of pre-switch duration allows an estimate of the relative effects of morphology plasticity and learning on foraging efficiency (fig. 5). The magnitude of the effect of learning on handling

efficiency (C/C versus C/G at *duration* = 72, fig.5[i-ii]) is much smaller than that of morphological plasticity (C/G versus G/G at *duration* = 72, fig. 5[i-ii]), suggesting that handling efficiency is predominantly effected by morphological plasticity. In contrast, learning has a large effect on both searching efficiency variables. Its effect appears roughly equal to that of morphological plasticity for search time/prey and only learning produced an effect on latency time (fig. 5[iii-iv]).

DISCUSSION

Recent theoretical explorations of the evolution of phenotypic plasticity reveal some ecological conditions under which plasticity should evolve (Via and Lande 1985; Gomulkiewicz and Kirkpatrick 1992; Gavrilets and Scheiner 1993a, 1993b), and a few empirical studies have attempted to correlate ecological factors and degree of plasticity (Wimberger 1991,1992). Some of these studies suggest that phenotypic plasticity can be adaptive; however, studies that quantify the adaptive significance of phenotypic plasticity are rare (Thompson 1992). Yet, such evidence is crucial to understanding the evolution of phenotypic plasticity. Further, even in instances where the adaptive significance of morphological plasticity has been measured, other possible explanations such as changes in behaviour usually have not been considered.

In my experiment with limnetic sticklebacks it took between 39 and 72 days of diet treatment to induce a detectable morphological change. The only morphological character that exhibited significant change was head depth, although gape width exhibited a consistent pattern of change when the duration of the diet treatment increased.

Comparison of the present results with those in chapter one suggest that diet-induced morphological changes may not be reversible but, instead, are fixed at some point in development (i.e., they are non-labile). Thus, the marginal diet-induced changes in only two of the four traits measured in this study may be a result of using wild fish that were already approximately 25mm - 30mm in standard length. Other differences between the two experiments, however, include the use of different prey items as well as a different total amount of growth achieved by fish in each experiment. Thus, an unequivocal explanation of the discrepancy cannot be given.

Diet-induced morphological changes probably result from plastic responses in either muscle or bone. Both substances can change size and shape under prolonged novel stresses (Lanyon 1984; Lanyon & Rubin 1985), especially if the stresses are imposed during critical periods in ontogeny (Wainwright et al. 1991; see Bertram & Swartz 1991 for discussion). In chapter one I suggested why mechanical stress is the probable proximate cause of plasticity rather than an alternative such as nutritional differences between diets.

Diet-induced changes in morphology were paralleled by changes in foraging efficiency (table 5; fig. 5). Additionally, head depth was significantly related to one of the handling efficiency variables (table 6). Although the functional utility of head depth is not known, this character probably is correlated with other, unmeasured morphological characters. Also, a large proportion of the variation in the efficiency variables was not explained by variation in the morphological characters measured. This implies that changes in unmeasured characters also must cause some of the divergence in efficiency. One

possible example is diet-induced change in neural morphology and the consequent effects on behaviour (Masai and Sato 1965; Krebs 1990; Healy et al. 1994). If neural morphology is more labile than the morphological characters measured, then low R^2 values for gape width and head depth may not represent the total effect of diet-induced morphological change.

The morphological characters chosen for study are the important (plastic) correlates of foraging efficiency in sticklebacks (Bentzen and McPhail 1984; Lavin and McPhail 1986; Schluter 1993) and therefore are a logical part of the anatomy to quantify. Unquestionably, however, other attributes of a fish's morphology also contribute to successful foraging. Thus, the divergence in foraging efficiency that I attribute to morphological plasticity probably is not solely due to diet-induced change in the characters examined. Rather, plasticity in these characters should be considered as an index of overall morphological plasticity.

Learning has a significant effect on search time/prey and appears to result in a consistent, though non-significant, effect on latency time. In contrast, learning does not have any discernible effect on either of the handling efficiency variables. This differs from the results of previous studies where learning affects both searching and handling efficiency (Werner et al. 1981; Dill 1983; Ehlinger 1989a, 1989b; Croy and Hughes 1991). The reason for this discrepancy between my data and earlier studies is not known.

Morphological Plasticity versus Learning. - The efficiency of the prey ingestion process is separable into two distinct components: searching efficiency, and handling efficiency. My results suggest that the effects of morphological plasticity and learning parallel this

division of foraging efficiency. The magnitude of the effect of learning on search time/prey is roughly equal to that of morphological plasticity and it appears to have had the only effect on latency time (fig. 5[iii-iv], at *duration* = 72 days). In contrast, the magnitude of the effect of morphological plasticity on both handling efficiency variables is substantially greater than that of learning (fig. 5[i-ii], at *duration* = 72 days). Several studies have demonstrated that learning affects both components of foraging efficiency but the present results are the first to my knowledge that compares the effects of learning with those of morphological plasticity.

Evolution Of Morphological Plasticity in Sticklebacks

In chapter one I demonstrated an association between diet variability and the degree of trophic morphological plasticity in two species of sticklebacks. Here I have demonstrated that this diet-induced morphological change is adaptive in that it increases foraging efficiency. Together these results provide some of the best evidence from natural populations to date suggesting that diet variability can drive the evolution of plastic trophic morphology. However, a few complicating factors remain to be addressed.

If morphological plasticity has evolved as a result of diet variability then it is important that the time scale of diet-induced morphological change is appropriately matched to the time scale of natural diet variability. I have shown that morphological changes take place on a time scale of 39 to 72 days. Observations suggest that the breeding season for the entire limnetic species' population in Paxton Lake is roughly 100 to 120 days (Schluter and McPhail 1992; pers. obs.). Although this is likely an

overestimate for the amount of time an individual spends breeding, it is a reasonable first approximation because most fish do not survive the winter to breed again the following year. Alternatively, plastic changes may occur more rapidly in the wild because fish are exposed to other environmental cues in addition to a change of diet (e.g., temperature, pH).

A potential objection to the above evolutionary hypothesis stems from the lack of substantial diet-induced morphological change in this study as compared to results of chapter one. The present results on diet-induced morphological change suggest that some of the traits considered might exhibit non-labile plasticity. If this is true, then the argument of *intra*-generation diet variability driving the evolution of morphological plasticity in these traits is not plausible (Gomulkiewicz and Kirkpatrick 1992; Scheiner 1993a). It is then curious why the limnetic species exhibits greater plasticity in these traits. A potential explanation resides in the differences in lifespan of the two species. Because individuals of the limnetic species have a shorter lifespan (approx. 1-2 years) than individuals of the benthic species (approx. 3+ years), the limnetic species may experience greater *inter*-generation resource variability than the benthic species. Non-labile plasticity could then be an adaptation to uncertain resource levels that are present in any particular generation. Yoshimura and Clark (1991), Gomulkiewicz and Kirkpatrick (1992), Scheiner (1993a) and Leon (1993) all provide discussions of adaptation to inter-generation variability. If this is true, then the effect of morphological plasticity on foraging efficiency that I have demonstrated may well be an underestimate. The full effect of morphological plasticity could only be demonstrated by administering the diet treatments early in ontogeny.

A final alternative hypothesis for the difference in degree of plasticity between the two species was suggested in chapter one. The limnetic species is morphologically less robust than the benthic species. For example, limnetic gillrakers are not only longer and more numerous than those of the benthic species, but are more slender as well. If less robust morphology is more susceptible to stress-induced change, this would explain how the interspecific difference in plasticity is realized. It is possible that differences in robustness have evolved as an incidental byproduct of evolutionary divergence in body form, and thus interspecific differences in plasticity are simply a (non adaptive) correlated response to selection on the mean value of each trait. At present it is not possible to distinguish between these alternatives.

Table 4. Results of the one-tailed univariate ANCOVAs for a diet-induced change in morphology and also the proportional changes calculated as $percent = 100 \bullet (trait_{Gam} - trait_{Cal}) / trait_{Cal}$. Only results from the third set are presented.

Morphological Character	$F_{1,44}$ (Diet)	Percent Change
Head Depth	6.10**	1.4
Gape Width	1.95 #	2.2
Gillraker Length	0.423	8.3
Snout Length	0.030	0.0

** $P < 0.01$, # $0.05 < P < 0.1$

Table 5. Results of the analysis for an effect of morphological plasticity and learning on foraging efficiency. Statistical model: $Y = \alpha + \beta \cdot duration + \delta_{pre} \cdot \gamma_{pre} \cdot duration^2 + \delta_{post} \cdot \gamma_{post}$. Indicator variables δ_{pre} and δ_{post} equal 1 (-1) if diet treatment is calanoids (gammarids). P-values are not Bonferroni corrected.

	$F_{1,87}$			Estimate			
Efficiency Variable	β	γ_{pre}	γ_{post}	α	β ($\cdot 10^{-3}$)	γ_{pre} ($\cdot 10^{-3}$)	γ_{post}
Handling time/Prey	0.888	12.9***	0.197	2.65	-3.98	0.111	0.0466
Attacks/Prey	0.104	13.2***	0.731	0.70	1.33	0.110	0.0876
Search time/Prey	0.944	4.49*	6.40**	1.58	5.88	0.0948	0.379
Latency time	0.516	0.199	4.40*	2.66	5.34	0.0240	0.388

*** $P < 0.001$, ** $P < 0.01$, * $P < 0.05$

Table 6. Results of the multiple regression analyses for the effect of morphological plasticity on foraging efficiency. N=124 for each.

	t-statistic	
Efficiency Variable	Head Depth	Gape Width
Handling time/Prey	-2.49**	0.61
Attacks/Prey	-1.68*	-0.51
Search time/Prey	-1.27	-0.22
Latency time	-0.62	0.39

** P < 0.01, * P < 0.05

Fig. 3. Design of the diet treatments. The pre-switch period is meant to induce morphological change and the post-switch period is meant to induce short-term behavioural change.

Fig. 4. Magnitude of the diet-induced change across the three sets plus/minus SE. Both morphological characters are size-corrected to a fish of 32.95 mm in standard length.

Fig. 5. The foraging efficiency variables across the three sets with regression lines calculated using estimates of table 2 as $Y = \alpha + \beta \bullet duration + \delta_{pre} \bullet \gamma_{pre} \bullet duration^2 + \delta_{post} \bullet \gamma_{post}$. Dashed (C/G) and solid lines (G/G) depict divergence of efficiency resulting from morphological plasticity and dotted line (C/C) reveals the change in efficiency resulting from learning relative to group C/G. Symbols are means of each group plus/minus SE; Δ=C/C, ▲=C/G, and ●=G/G.

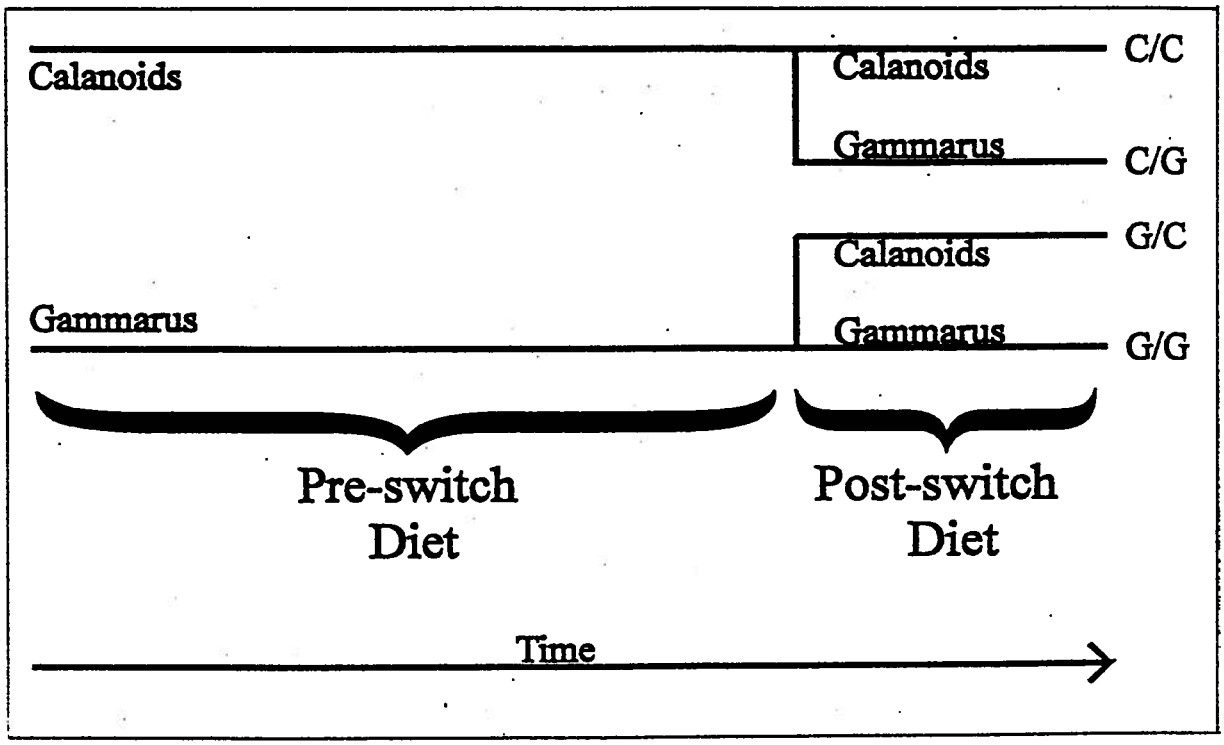

Calanoids
Gammarus
Calanoids
Gammarus
Calanoids
Gammarus
C/C
C/G
G/C
G/G
Pre-switch Diet
Post-switch Diet
Time

Figure 3

Figure 4

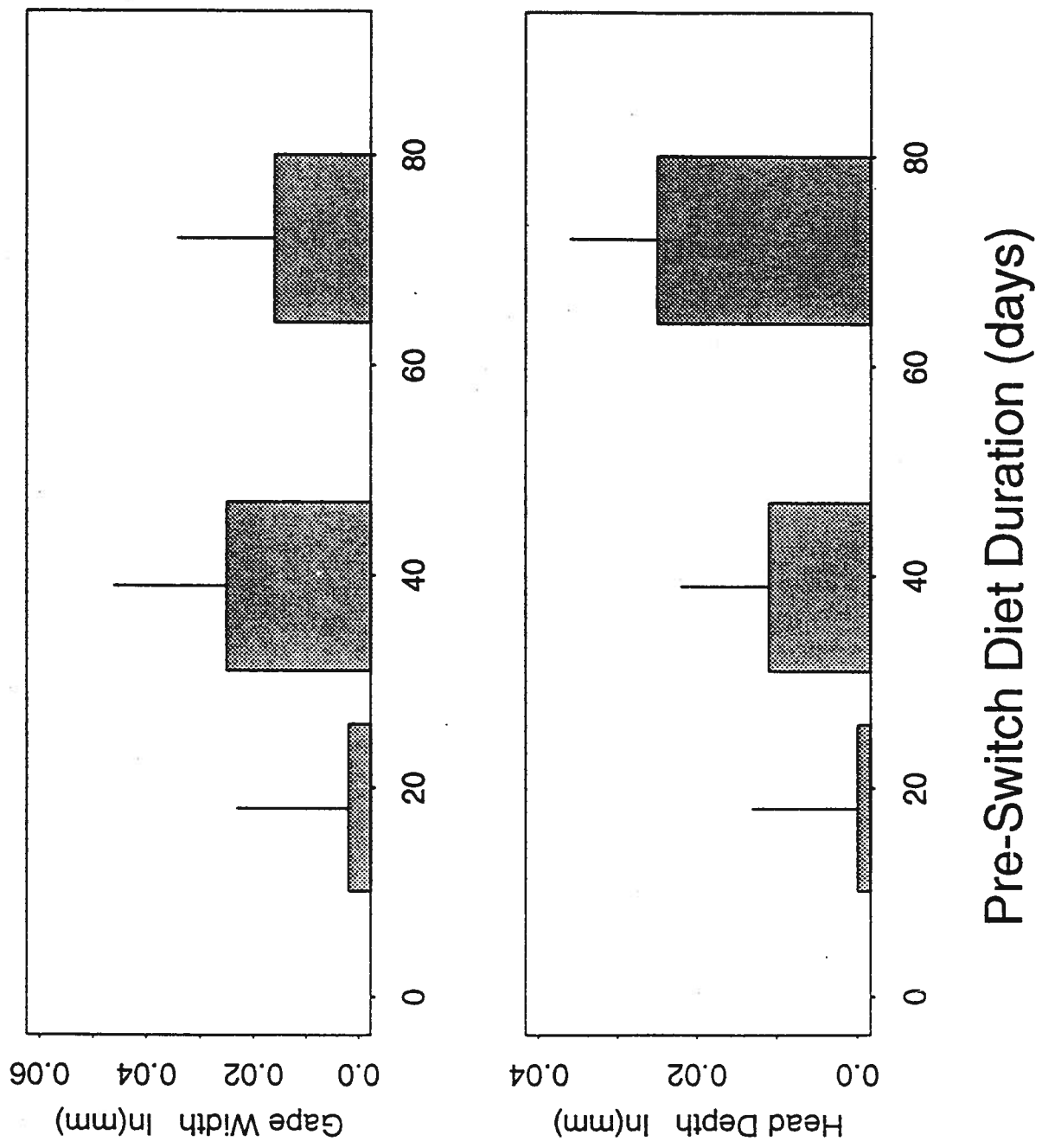
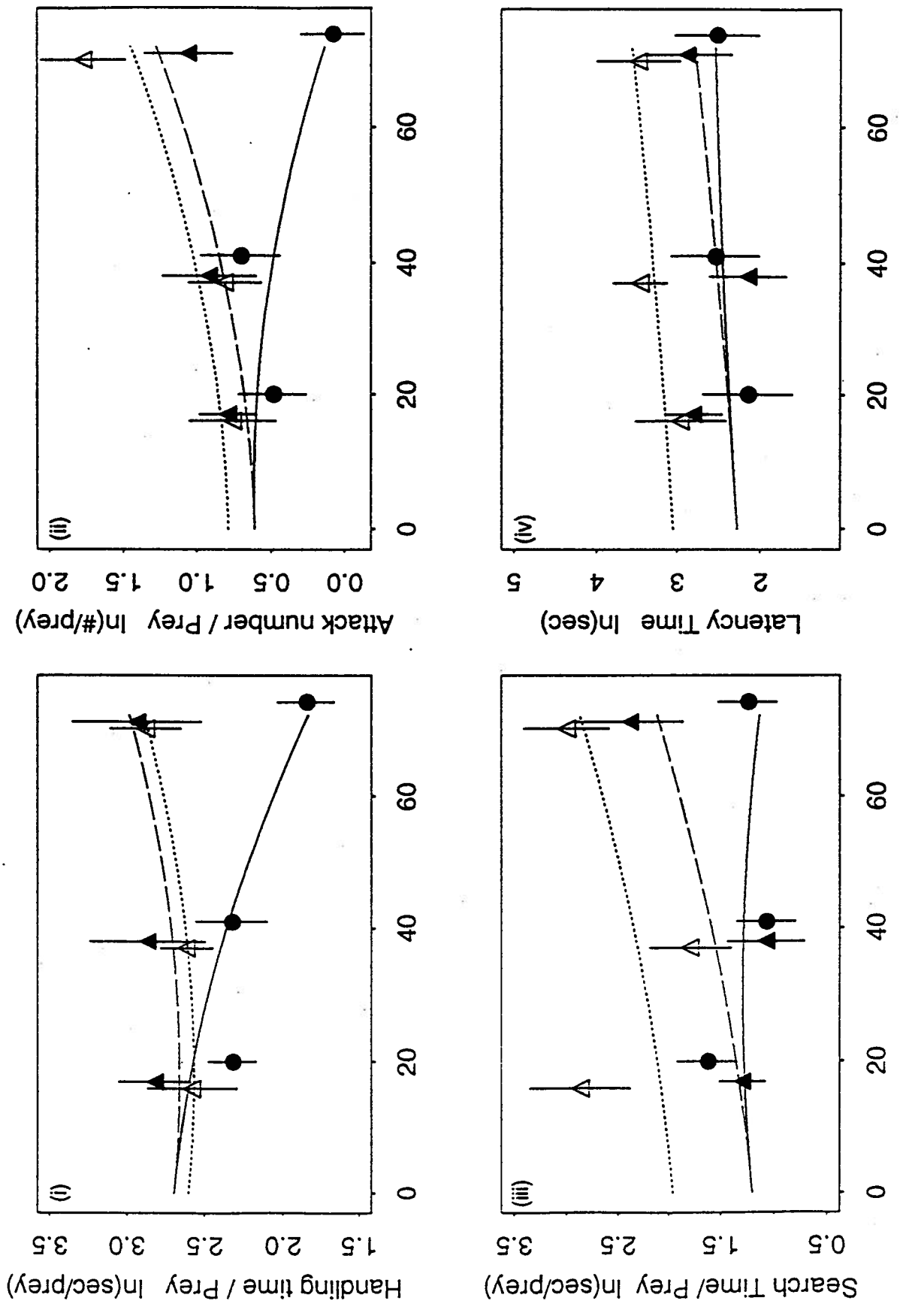

Diet-Induced Change
Gape Width ln(mm)
0.0
0.02
0.04
0.06
Head Depth ln(mm)
0.0
0.02
0.04
0
20
40
60
80
Pre-Switch Diet Duration (days)

Figure 5


Pre-Switch Diet Duration (days)
Search Time/ Prey ln(sec/prey)
Latency Time ln(sec)
Handling time / Prey ln(sec/prey)
Attack number / Prey ln(#/prey)
(iii)
(iv)
(ii)
(i)
0.5
1.5
2.5
3.5
2
3
4
5
1.5
2.0
2.5
3.0
3.5
0.0
0.5
1.0
1.5
2.0
0
20
40
60

GENERAL DISCUSSION

In chapter one I demonstrated an association between diet variability and the degree of trophic morphological plasticity in two species of stickleback. I also provided evidence suggesting that morphological plasticity is adaptive and demonstrated that morphological plasticity is heritable. These results are some of the best evidence from a natural population to suggest that diet variability can drive the evolution of plastic trophic morphology.

In chapter two I extended these results by demonstrating that diet-induced morphological change is adaptive in that it increases foraging efficiency. I also provided evidence revealing that the time scale of morphological change is roughly compatible with the time scale of natural resource variability. Together, the results from both chapters provide strong support for the proposed evolutionary hypothesis. However, it is important to emphasize the complications discussed in chapters one and two. The alternative hypotheses mentioned there require a more in depth treatment before they can be completely dismissed. Additionally, it would be useful to have a clear understanding of the proximate mechanism whereby diet-induced changes are realized.

In addition to addressing issues regarding the evolution of morphological plasticity, the results of these two experiments suggest ways in which individual fish adapt to resource variability. Short-term conditioning on a particular prey largely results in behavioural change which increases searching efficiency. Long-term conditioning on a particular prey results in behavioural and morphological change which increases both

searching and handling efficiency. These mechanisms result in adaptation to environmental conditions on time scales shorter than those usually envisaged for evolutionary change. Additionally, the present results reveal that substantial adaptation of a population can take place without any genetic change.

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