Oecologia (1996) 108:380–388

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The effect of behavioural and morphological plasticity on foraging efficiency in the threespine stickleback (*Gasterosteus* sp.)

Received: 26 April 1995 / Accepted: 22 April 1996

Abstract We conducted an experiment to assess the change in foraging efficiency resulting from diet-induced morphological and behavioural plasticity in a species of freshwater, threespine stickleback (*Gasterosteus* sp.). Different degrees of morphological and behavioural change were induced using two prey items commonly found in the diet of this species, allowing us to estimate the relative importance of each type of plasticity. The purpose of the experiment was twofold. First, earlier work had suggested that diet variability might be an important factor in the evolution of trophic morphological plasticity in sticklebacks. The present results extend this work by revealing the adaptive significance of morphological plasticity. The current experiment also qualitatively assessed the compatibility of the time scale of morphological change with that of the natural resource variability experienced by this species. The results indicate that diet-induced plasticity improves foraging efficiency continuously for up to 72 days of prey exposure. This is probably due in part to plasticity of the external trophic morphology but our results also suggest a complex interplay between morphology and behaviour. The time scale appears to be matched to that of natural diet variability although it is possible that some traits exhibit non-labile plasticity. Our discussion highlights the important distinction between conditions favouring the evolution of labile versus non-labile plasticity. The second objective of the experiment was to determine the relative importance of morphological and behavioural plasticity. Few studies have attempted to quantify the adaptive significance of morphological plasticity and no study to our knowledge has separated the effects of morphological and behavioural plasticity. Our experiment reveals that both behavioural and morphological plasticity are important and it also suggests a dichotomy between the two: behavioural plasticity predominately affects searching efficiency whereas morphological plasticity predominately affects handling efficiency.

Key words Adaptive significance of plasticity · Behavioural versus morphological plasticity · Phenotypic plasticity · Foraging efficiency · Sticklebacks

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Introduction

Many organisms can modulate their phenotype in response to environmental cues. Such plasticity can often be an important adaptive strategy for coping with environmental variability (Stearns 1989; Scheiner 1993). Additionally, it is likely that phenotypic plasticity plays an important role in the process of diversification (West-Eberhard 1989). Interest in the evolution of phenotypic plasticity has resulted in significant advances in theoretical research (Levins 1968; Via and Lande 1985; Lively 1986; Gomulkiewicz and Kirkpatrick 1992; Leon 1993; Gavrilets and Scheiner 1993a, b), but 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; Meyer 1987, 1990; Dodson 1989; van Noordwijk 1989; Harvell 1990; Witte et al. 1990; Wimberger 1991, 1992; Day et al. 1994), most empirical tests of theories concerning the evolution of phenotypic plasticity have come from laboratory studies of *Drosophila* (Scheiner 1993). 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 paper we extend previous work that suggested diet variability can drive the evolution of plastic trophic morphology. Day et al. (1994) demonstrated an association between diet variability and the degree of diet-induced morphological plasticity in two morphs of the freshwater threespine stickleback (*Gasterosteus* sp.). Ad-

ditionally, they documented the heritability of morphological plasticity and provided evidence which suggests diet-induced morphological change may be adaptive. The present work complements these results in three ways. First, we examine the time-scale over which diet-induced morphological change occurs to determine if it is compatible with the time-scale of diet variability. Second, we examine the effect of diet-induced morphological plasticity on foraging efficiency. Third, we examine the effect of short-term behavioural plasticity on foraging efficiency and compare its importance to that of morphological plasticity.

Theoretical work suggests that the extent to which plasticity evolves depends upon the rate at which the phenotype can respond 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 of those that exist (e.g. Thompson 1992; Robinson and Wilson 1995), few consider the potential effect of diet-induced behavioural change. Yet, behavioural plasticity has a large impact on foraging efficiency (Werner et al. 1981; Dill 1983; Ehlinger 1989a, b, 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 are often confounded.

The present study was designed to examine both types of phenotypic plasticity. We define behavioural plasticity as the behavioural change that takes place on a time-scale short enough to preclude most morphological change. The effect of behavioural plasticity 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, resulting in indirect behavioural changes (Krebs 1990; Healy et al. 1994 and references therein). Furthermore, behavioural plasticity 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. Therefore, we 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 prey exposure. This effect is measured by documenting changes in foraging efficiency that take place with long-term exposure to prey.

Plasticity and sticklebacks

The study organism is a morph of the freshwater threespine stickleback (*Gasterosteus* sp.) found in four coastal drainages of British Columbia, Canada (McPhail 1993). It is termed the "limnetic" morph because it forages predominantly on calanoid copepods (*Diaptomus* sp.) in the "limnetic" (water column) habitat of lakes. The limnetic morph coexists with a "benthic" morph which forages predominantly on invertebrates (gammarids) in the littoral region of lakes. The two morphs are morphologically distinct, and differences in morphology have significant effects on foraging efficiency and growth in the two habitats (Bentzen and McPhail 1984; Schluter 1993, 1995). The limnetic morph has a long snout, long gill-rakers, a slender head and a narrow gape relative to the benthic morph (McPhail 1992). These morphological differences are largely heritable (McPhail 1992; Day et al. 1994) and are thought to have evolved as a result of competition for resources and character displacement (Schluter and McPhail 1992, 1993).

Day et al. (1994) have demonstrated that the limnetic morph exhibits greater diet-induced morphological plasticity than the benthic morph. Because individuals of the limnetic morph forage in the littoral habitat during their breeding season (Schluter and McPhail 1992; Schluter 1993), they experience substantial resource variability. In contrast, benthic fish forage in the littoral habitat throughout their lives and therefore have a relatively monotonous diet. Therefore, Day et al. (1994) suggested that greater diet-induced morphological plasticity in the limnetic species may have evolved to cope with this high degree of resource variability.

Here we document the time-scale over which morphology changes, by keeping limnetic fish 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 us to determine if the two are compatible. Also, by conducting foraging trials on gammarid prey items with fish from these two diet treatments, we can quantify the effect of morphological and behavioural plasticity. If diet-induced morphological plasticity has evolved as a result of diet variability, then limnetic fish with gammarid-induced morphology should be more efficient foragers on gammarids than limnetic fish with calanoid-induced morphology (all else being equal).

Materials and methods

Limnetic fish were taken from Paxton Lake, British Columbia, Canada in mid-April 1993 using dip-nets. Each individual (25–30 mm standard length) was randomly assigned to a diet of calanoid copepods (*Diaptomus* sp.) or gammarid amphipods (*Gammarus lacustris*). These diet treatments represent prey commonly found in the natural foraging habitats of the limnetic morph (Schluter and McPhail 1992). Fish were kept in eight 102-l aquaria (four per diet treatment) at an initial density of 30 individuals per aquarium. Density declined throughout the summer as a result of mortality and sampling for foraging trials. All fish that died were kept for comparison with surviving individuals to check that morphological differences between diet treatments resulted from diet-induced change rather than differential mortality. The photoperiod was held constant (16 h light, 8 h dark) and temperature varied with ambient temperature (13–19°C).

Diet treatment

We used a diet-switching experimental design (Fig. 1). The pre-switch period was longest and was meant to induce an effect of both behavioural and morphological plasticity on foraging efficiency. The shorter post-switch period was meant to induce only an effect of behavioural plasticity. Foraging efficiency on gammarid prey was assessed at the end of the post-switch period.

There were three durations of pre-switch diet exposure (termed sets 1, 2 and 3, with 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 10 days for all three sets. Although the duration of the post-switch period is somewhat arbitrary, 10 days is probably sufficient to produce efficiency changes through learning (Werner et al. 1981; Ehlinger 1989a; Schluter 1993), yet short enough to preclude most morphological change (see Discussion).

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, the remainder being given the diet opposite to that experienced during the pre-switch period. Each fish was kept individually in a 10-l aquarium for the entire 10-day post-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. 1).

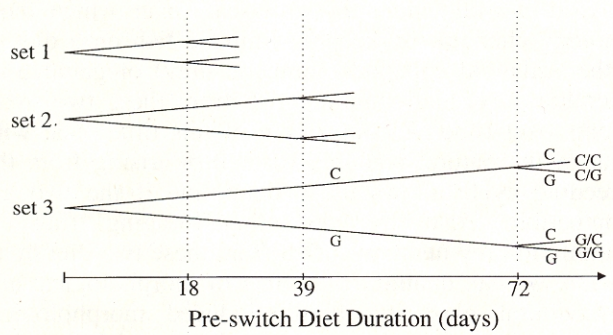

Fig. 1 Design of the diet treatment. The pre-switch period was 18, 39 or 72 days in duration and was meant to induce morphological change. The post-switch period was 10 days for each set and was meant to induce short-term behavioural change (*C* Calanoids, *G* Gammarids)

Foraging trials

Foraging trials were used to determine how experience with gammarids or copepods affects efficiency of foraging on gammarids. In each trial, a single fish was placed in a 102-l aquarium containing ten gammarids [mean length = 3.47 mm (SD = 0.53), mean width = 1.06 mm (SD = 0.18)] on a sand substrate. We 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 10 min or the consumption of two prey items, whichever came first. Each fish was sacrificed after its foraging trial so that morphological measurements could be made.

Measures of foraging efficiency and morphology

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

Searching efficiency measures are (1) time from fish introduction to first strike at prey (latency time) and (2) search time/prey. Search time/prey was calculated by dividing total search time (i.e. time spent searching substrate or following prey) by the number of prey consumed plus one. One was added to the denominator to prevent division by zero where a fish searched for, but did not consume, prey items. Both variables were log-transformed to normalize their distributions.

Handling efficiency measures are (3) handling time/prey and (4) number of strikes/prey. Both were calculated by dividing total handling time (i.e. total time spent manipulating prey), or total number of strikes, 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) gill-raker length, (2) head depth (measured at the posterior edge of the operculum), (3) gape width, (4) snout length (distance from tip of snout to eye) and (5) standard length. Characters 1–4 were chosen because they exhibit a plastic response to diet (Day et al. 1994), and because they form part of a suite of morphological structures whose dimensions provide a reliable index of habitat specialization in freshwater fishes (Lindsey 1981; Bentzen and McPhail 1984; Lavin and McPhail 1986; Schluter 1993; Schluter and McPhail 1993; Robinson and Wilson 1994). Although other morphological characters probably exhibit diet-induced change, those we chose provide a useful index of overall trophic morphology. Character 5 was used as a covariate for size correction of various measurements. All measurements were log-transformed to equalize variances. Characters 1–4 were measured using an ocular micrometer at 6.4x - 16x magnification and 5 was measured using Vernier calipers.

Size-corrected morphological characters were used in all analyses unless otherwise stated. Size correction was carried out by least-squares regression of each character against standard length using combined data from all three pre-switch durations (sets). Residuals of these regressions were added to the mean of the combined data.

Analysis

Most of the analyses are one-tailed. The analysis for diet-induced morphological change is one-tailed because previous work (Day et al. 1994) demonstrated a consistent directionality 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, table-wide significance levels are corrected using the sequential Bonferroni technique (Rice 1989). All statistical analyses are performed using Systat 5.02 for IBM (Wilkinson et al. 1992).

To rule out differential mortality as a cause of observed morphological change, we conducted a one-way MANOVA (Johnson and Wichern 1982) on the fish that died. We used pre-switch diet treatment as the independent variable and the morphological characters that exhibited change as the dependent variables. If differential mortality caused 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 on the morphology of dead fish 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 change had occurred in both the fish that died and those that survived. Therefore, morphological changes are not likely to be the result of differential mortality.

Time-scale

An assumption in the experimental design is that most diet-induced morphological change occurs on a time-scale longer than 10 days. This assumption was tested (for the measured characters) 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 (with a non-directional alternative hypothesis) on the four morphological variables. The same procedure was used with treatments G/C and G/G. The results of these tests confirm that 10 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, we 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 we had two "morphology" groups from each pre-switch duration to compare (group being determined by pre-switch diet treatment, Fig. 1). 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. Although this duration may be an overestimate as a result of type II error, it is a reasonable approximation because three temporal measurements allows only a crude resolution of the time-scale.

Effect of morphological and behavioural plasticity

Treatments C/C, C/G and G/G (Fig. 1) were used to estimate the effect of morphological and behavioural plasticity. 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 behavioural plasticity was tested by comparing group (C/C) with (C/G + G/G) because these two groups differ in post-switch diet.

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

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

Y is the measure of foraging efficiency and duration is the length of pre-switch diet duration 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 the efficiency of the two pre-switch diet groups [(C/C + C/G)] versus [(G/G)] diverges non-linearly with time (represented by the term $\gamma_{pre} \cdot \delta_{pre} \cdot \text{duration}^2$); the quadratic was a good approximation to this non-linear divergence (see Results). 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 and 0.038 for attacks/prey, handling time/prey, search time/prey and latency time, respectively), the effect of behavioural plasticity 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 ($\beta \cdot$ duration). If γ_{pre} or γ_{post} is significantly different from zero, foraging efficiency was affected by morphological plasticity or behavioural plasticity, respectively.

As an additional analysis for the effect of morphological plasticity, we conducted a univariate multiple regression for each efficiency variable using those 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 combined from all three pre-switch durations.

Results

Time-scale

Head depth was the only morphological character that exhibited significant diet-induced change, and then only in the longest pre-switch duration (Table 1). None of the characters exhibited statistically significant plasticity at a shorter pre-switch duration, although the trend in gape width appeared to plateau (or even decline) after the sec-

Table 1 Results of the one-tailed univariate ANCOVAs for a diet-induced change in morphology. The columns trait_{Cal} and trait_{Gam} are the trait means on the two diet treatments. Values are calculated using log-transformed measurements, size-corrected to a fish of 32.95 mm in standard length. Also shown are the proportional changes calculated as $100 \cdot (\text{trait}_{Gam} - \text{trait}_{Cal})/\text{trait}_{Cal}$ Only results from the third set (morphology at 72 days) are presented

Morphological character	Diet treatment		Change %	$F_{1,44}$ (diet)
	trait_{Cal} (SE)	trait_{Gam} (SE)		
Head depth	1.791 (0.005)	1.816 (0.009)	1.4	6.10**
Gape width	0.716 (0.015)	0.732 (0.012)	2.2	1.95*
Gill-raker length	–0.084 (0.021)	–0.091 (0.02)	8.3	0.423
Snout length	1.152 (0.012)	1.152 (0.015)	0.0	0.030

* $0.05 < P < 0.1$; ** $P < 0.01$

ond set (Fig. 2). 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 change in gill-raker length or snout length across the three pre-switch durations. These results differ from those of a previous study (Day et al. 1994), which demonstrated substantial plasticity in all four morphological characters. Fish were kept on different diets from an earlier age in the previous study, however, which suggests that some traits might exhibit non-labile plasticity. It is also possible that differences in diet and/or growth rate between the present and previous experiments account for the difference in plasticity (see Day et al. 1994).

Effect of morphological plasticity

Foraging efficiency on gammarids improved continuously as an individual's experience with gammarids increased up to 72 days; all measures of foraging efficiency, except latency time, exhibited significant divergence between the two pre-switch diet treatments [i.e. γ_{pre} is significantly different from zero, Table 2; C/C (and C/G) diverge from G/G as pre-switch duration increases, Fig. 3]. This divergence of foraging efficiency between the two treatments parallels the morphological divergence between these two treatments (Fig. 2). This suggests that part of the change in efficiency may be due to plasticity in the characters examined.

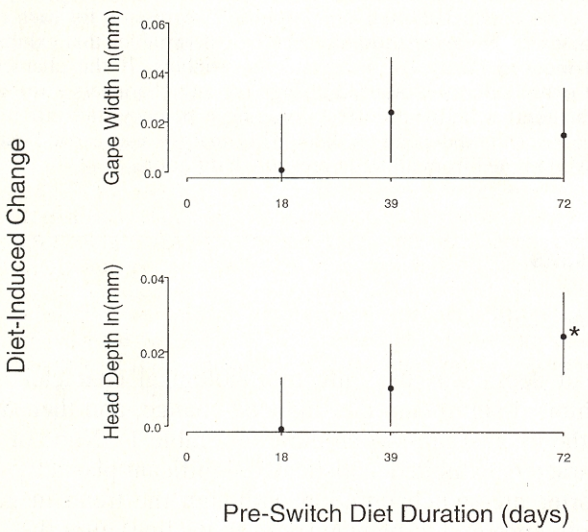

Fig. 2 Magnitude of the diet-induced change across the three sets ± SE. Both morphological characters are size-corrected to a fish of 32.95 mm in standard length. Diet-induced changes are calculated as $trait_{Gam}$ - $trait_{Cal}$. (* differs from zero, $P < 0.05$)

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 the same pattern.

Multiple regression analyses of specific morphological characters reveal that only head depth is significantly related to handling time/prey (Table 3). 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), which implies much variation in foraging efficiency which is 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 behavioural plasticity

Both searching efficiency variables were improved by post-switch experience with gammarids (γ_{post} for latency time, P = 0.02; γ_{post} for search time/prey, P = 0.007; Table 2) but, using the sequential Bonferroni technique, only the effect on search time/prey was significant. This variable displayed both an effect of morphological plasticity and an effect of behavioural plasticity (Table 2, Fig. 3iii). Lack of a significant effect of behavioural plasticity 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. 3iv). Neither handling efficiency variable exhibited a significant effect of behavioural plasticity nor did they display any consistent pattern at each pre-switch duration.

Table 2 Results of the analysis for an effect of morphological plasticity and behavioural plasticity 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)

Efficiency variable	$F_{1,87}$			Estimate			
	β	γ_{pre}	γ_{post}	β	α ($\cdot 10^{-3}$)	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<0.05; ** P<0.01; *** P<0.001

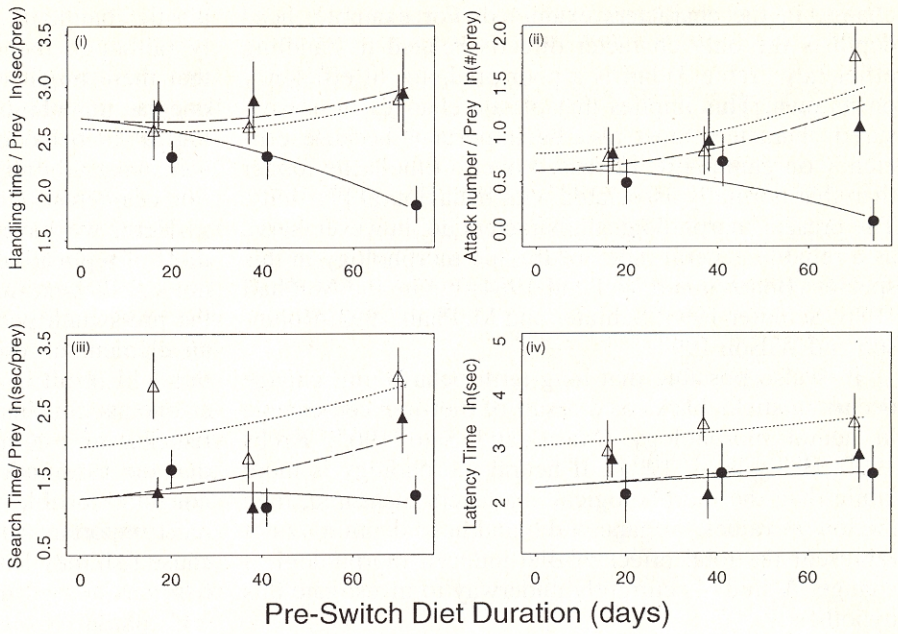

Fig. 3 The foraging efficiency variables across the three sets with regression lines calculated using estimates of table 2 as $Y = \alpha + \beta \cdot \text{duration} + \delta_{pre} \cdot \gamma_{pre} \cdot \text{duration}^2 + \delta_{post} \cdot \gamma_{post}$. Foraging efficiency refers to foraging on gammarids. *Dashed* (C/G) and *solid lines* (G/G) depict divergence of efficiency resulting from morphological plasticity and the *dotted line* (C/C) reveals the change in efficiency resulting from behavioural plasticity relative to group C/G. Symbols are means of each group ± SE: △ C/C, ▲ C/G and ● G/G

Table 3 Results of the multiple regression analyses for the effect of morphological plasticity on foraging efficiency. Coefficients are the slope parameters of the multiple regression for each efficiency variable. n=124 for each

Efficiency variable	Coefficient	
	head depth	gape width
Handling time/prey	−5.57**	0.85
Attacks/prey	−3.84*	−0.73
Search time/prey	−3.77	−0.41
Latency time	1.77	−1.91

* $P<0.05$; ** $P<0.01$

A comparison of the elevations of the three regression lines at 72 days pre-switch duration allows an estimation of the relative effects of morphological plasticity and behavioural plasticity on foraging efficiency (Fig. 3). The magnitude of the effect of behavioural plasticity on handling efficiency (C/C vs. C/G at duration = 72; Fig. 3i, ii) is much smaller than that of morphological plasticity (C/G vs. G/G at duration = 72; Fig. 3i, ii), suggesting that handling efficiency is predominantly affected by morphological plasticity. In contrast, behavioural plasticity 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 behavioural plasticity produced an effect on latency time (Fig. 3iii, iv).

Discussion

Diet-induced morphological changes probably result from plastic responses in either muscle or bone. Both substances can change size and shape under prolonged novel mechanical stress (Lanyon 1984; Lanyon and Rubin 1985), especially if the stress is imposed during critical periods in ontogeny (Wainwright et al. 1991; see Bertram and Swartz 1991 for discussion). Day et al. (1994) suggest that mechanical stress arising from prey-specific foraging modes is the proximate cause of plasticity in sticklebacks.

It took between 39 and 72 days of diet treatment to induce a detectable morphological change in limnetic sticklebacks. Head depth changed continuously for the entire 72 days and change in gape width appeared to plateau somewhere between 39 and 72 days. Comparison of the present results with those of a previous study (Day et al. 1994) suggests 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 smaller diet-induced changes in two of the four traits measured in this study may be a result of using wild fish that were already approximately 25–30 mm in length. It is possible, however, that diet-induced change occurs slowly and, therefore, a longer diet exposure is required to induce greater morphological change. 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.

Diet-induced changes in morphology were paralleled by changes in foraging efficiency (Table 2, Fig. 3). 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; Day et al. 1994) and are therefore a logical part of the anatomy to examine. Unquestionably, however, other attributes of fish morphology also contribute to successful foraging. Thus, the divergence in foraging efficiency that we attribute to morphological plasticity is probably not solely due to diet-induced

change in the characters examined. For example, head depth is the only character directly related to handling efficiency (Table 3) but is a poor predictor of efficiency on its own. This implies that plastic changes in unmeasured characters must also be important, because efficiency on gammarids increased over a time scale longer than that normally associated with behavioural plasticity. The present morphological characters do, however, serve as a reliable general index of trophic morphology in this species (Bentzen and McPhail 1984; Lavin and McPhail 1986; Schluter 1993; Schluter and McPhail 1993; Robinson and Wilson 1994).

It is also possible that long-term behavioural change occurs in sticklebacks as a result of diet-induced change in neural morphology (Masai and Sato 1965; Krebs 1990; Healy et al. 1994). If neural morphology is more labile than the morphological characters measured, then the low r^2 values for gape width and head depth may not represent the total effect of diet-induced morphological change. A study is currently underway to investigate this hypothesis.

Behavioural plasticity has a significant effect on search time/prey and appears to result in a consistent, though non-significant, effect on latency time. In contrast, behavioural plasticity 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, b; Croy and Hughes 1991).

Morphological plasticity versus behavioural plasticity

The efficiency of the prey ingestion process is separable into two distinct components: searching efficiency and handling efficiency. Our results suggest that the effects of morphological plasticity and behavioural plasticity parallel this division of foraging efficiency. The magnitude of the effect of behavioural plasticity on search time/prey is roughly equal to that of morphological plasticity and it appears to be the only effect on latency time (Fig. 3iii, 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 behavioural plasticity (Fig. 3i, ii; at duration = 72 days). Several studies have demonstrated that behavioural plasticity affects both components of foraging efficiency, but the present results are the first to our knowledge that compare the effects of behavioural plasticity with those of morphological plasticity.

It might be argued that the present results do not adequately depict the effects of behavioural and morphological plasticity separately because there could be long-term behavioural change and/or short-term morphological change in unmeasured characters. While this possibility does exist, our results do reveal the effects of long-term versus short-term plasticity. These two time-scales of plastic change generally parallel the time-scales of plastic change in morphology and behaviour, respectively (although there are exceptions). Additionally, given that there might not be any rigorous method by which one can unambiguously label any trait as either morphological or behavioural, the delineation we make, albeit somewhat arbitrary, captures a large part of the distinction of interest.

Here, we have considered individual fish to be the units of replication, but in our experimental design this is not strictly true since community tanks were used during the pre-switch period. Therefore, our analysis embodies an element of pseudoreplication. The extent to which this will result in erroneous significance levels and thus erroneous conclusions depends on the degree to which fish sharing a common tank are independent. We suggest that the assumption of independence in our analysis is not unreasonable. Community tank effects are probably most important with respect to behavioural plasticity because fish may learn by watching their tank mates. Each fish was housed in its own aquarium during the "learning" phase of our experiment, however, and therefore problems using community aquaria pertain mostly to measures of morphological plasticity. It is probably reasonable to assume that the diet-induced morphological changes of tank mates are at least approximately independent, especially since fish used in our experiment had already attained 25–30 mm in standard length in the wild. Additionally, the pattern of morphological plasticity which we document is consistent with previous results (Day et al. 1994), thus lending credibility to the inferences drawn here.

Evolution of morphological plasticity in sticklebacks

Previous work (Day et al. 1994) has demonstrated an association between diet variability and the degree of trophic morphological plasticity in two morphs of sticklebacks. Here we 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 to date from natural populations 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 the time-scale of diet-induced morphological change would probably be appropriately matched to the time-scale of diet variability. We have shown that morphological changes take place over 39–72 days. Observations suggest that the breeding season for the entire limnetic morph population in Paxton Lake is roughly 100–120 days (Schluter and McPhail 1992, personal observation). Although this is probably an overestimate of the amount of time an individual spends breeding, it is a reasonable 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 envi-

ronmental 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 previous results. The present results 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 1993; Day et al. 1994). It is then curious why the limnetic morph exhibits greater plasticity in these traits. A potential explanation resides in the differences in life-span of the two morphs. Because individuals of the limnetic morph have a shorter life-span (approx. 1–2 years) than individuals of the benthic morph (approx. 3+ years), the limnetic morph may experience greater inter-generation resource variability than the benthic morph. Non-labile plasticity could then be an adaptation to variability in the type of resources present across generations. Yoshimura and Clark (1991), Gomulkiewicz and Kirkpatrick (1992), Scheiner (1993) 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 we 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 morphs was suggested by Day et al. (1994). The limnetic morph is morphologically less robust than the benthic morph. For example, limnetic gill-rakers are not only longer and more numerous than those of the benthic morph, but are slenderer as well. If a less robust morphology is more susceptible to stress-induced change, this would explain how differences in plasticity are realized. Difference in robustness could possibly have evolved as an incidental by-product of evolutionary divergence in body form; thus, differences in plasticity would simply be 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.

Acknowledgements We appreciate help from L. Nagel with all aspects of the experiment. S. Heard, L. Nagel and D. Schluter were sources of innumerable helpful editorial suggestions and D. Schluter clarified our data analysis significantly. We are also grateful to P. Abrams and W. E. Neill for discussions on the subject and D. H. Wise and an anonymous reviewer for several important suggestions that clarified the presentation of these results. This work was funded by a Natural Sciences and Engineering Research Council of Canada grant to J.D.M.

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