Evolutionary Ecology Research, 2005, **7**: 801–819

Trophic plasticity and fine-grained resource variation in populations of western mosquitofish, *Gambusia affinis*

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ABSTRACT

Hypothesis: Fish will exhibit morphological plasticity in response to *how* (food type: live vs. attached food) and *where* (orientation: surface, mid-water, benthic) they are fed. Fine-grained resource variation (daily rotation of orientations) will produce intermediate morphologies. Population differences will reflect responses to both predators and resources.

Organism: Offspring from two populations of western mosquitofish (*Gambusia affinis*).

Experiment: A factorial design of 16 treatments: 4 (3 static orientations + 1 rotating diet) × 2 (food types) × 2 (populations) replicated three times.

Methods: We raised fish in the different diet treatments for 60 days. Morphology was characterized using geometric morphometric techniques. Multivariate analysis of covariance, visualizations and multiple regressions were used to assess morphological variation.

Conclusions: Responses to *how* and *where* fish fed resembled general morphological paradigms across species and may be adaptive. Fine-grained resource variation produced intermediate and unique shape effects. Population differences appeared to be related to trophic and predation ecology.

Keywords: ecomorphology, fine-scale resource variation, foraging ecology, geometric morphometrics, induced morphology, population differentiation, resource polymorphism, trophic plasticity.

INTRODUCTION

Natural environments vary spatially and temporally at myriad scales (Schmalhausen, 1949; Levins, 1968). This variation poses a problem for organisms because trait optima can differ between environments. Such trade-offs constitute divergent natural selection. This may lead to the evolution of phenotypic plasticity, population differentiation, ecological generalization or bet-hedging depending on the spatial and temporal pattern of environmental variation and gene flow (Levins, 1968; Sultan and Spencer, 2002; DeWitt and Langerhans, 2004). Generally, phenotypic diversification is greatest when the grain of environmental variation is relatively

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coarse (Levins, 1968). Coarse spatial variation drives genetic divergence among populations, whereas coarse temporal variation (i.e. between generations) favours the evolution of phenotypic plasticity (Levins, 1968; Berrigan and Scheiner, 2004). Plasticity is also expected to evolve in response to fine-grained environmental variation, such as seasonality in annual organisms, if phenotypic responses can keep pace with environmental change (Bradshaw, 1965; Padilla and Adolph, 1996). For example, facultative behaviours may be especially useful under fine-grained temporal variation because behaviour can be changed rapidly (Silvertown, 1998; Sih, 2004). However, it is still an open question how developmental plasticity may be adaptive under fine-grained temporal variation where the pace of developmental change cannot be expected to match that of environmental change.

Developmental plasticity has been studied as an adaptive solution to variation on several environmental gradients, including type or quantity of resources, predation risk, competition and abiotic stress (West-Eberhard, 1989; Schlichting and Pigliucci, 1998). Most of these gradients vary on multiple scales. Even where a gradient remains objectively constant, variation in individual behaviour may create subjective (experiential) environmental variance. For example, zooplankton exist as a constant potential food source in open water for juvenile sunfish (*Lepomis* spp.), but when large piscivorous fish are present juvenile sunfish remain in weedy refugia and consume alternative resources (Werner *et al.*, 1983). Thus, an objective gradient (predators) may cause subjective change experienced by focal organisms on another (resource) gradient. If there is plasticity in response to the second gradient, one might expect interesting cross-domain interactions such as 'trait-mediated indirect plasticity'. Such cross-domain interactions are likely to be common in natural systems, but this too is an open question at present.

Among the gradients mentioned above, resource variation is particularly apt for studies of evolutionary diversification (Smith and Skulason, 1996). Resources typically vary on many spatial and temporal scales (e.g. Keast and Webb, 1966). Additionally, extrinsic factors can influence patterns of resource use, particularly biotic interactions such as competition and predation, or abiotic factors such as oxygen or chemical gradients (e.g. Werner *et al.*, 1983; Olson *et al.*, 1995; Chapman *et al.*, 2000; Relyea and Mills, 2001). Phenotypic polymorphism associated with resource variation is well known in fishes (reviewed in Robinson and Wilson, 1994; Smith and Skulason, 1996). Resource polymorphism often results from both plastic and genetic factors working in concert to generate diversification (Day *et al.*, 1994; Robinson and Wilson, 1994; Robinson and Parsons, 2002). Resource polymorphism in fishes is also an apt system for study because strong paradigms exist for interpreting the functional consequences of morphological variation (Keast and Webb, 1966; Barel, 1983; Winemiller, 1991; Svanback and Eklov, 2003).

In this study, we examined trophic plasticity at different scales of resource variation in western mosquitofish, *Gambusia affinis*. We created multiple foraging treatments (alternative food types offered in alternative orientations), including a treatment that simulated temporally fine-grained resource variation (daily rotation of feeding orientation). Our primary goal was to determine if plasticity can produce intermediate (possibly generalist) phenotypes in addition to its classically understood role of producing relatively specialized morphologies across temporally coarse-grained environmental gradients. Additionally, two populations were used to add a spatially coarse-grain component of variation. The populations were chosen to have alternative predation regimes so we could address, in at least a preliminary manner, the intersection of two domains (predation and trophic ecology) in a plasticity study. We expected plasticity in response to food to be similar to that observed by Robinson and Wilson (1995) for guppies. We predicted fixed morphological differences between populations with contrasting predation regimes to reflect both direct effects of predators (*sensu* Langerhans *et al.*, 2004) and indirect effects of altered habitat and food use (*sensu* Robinson & Wilson, 1995).

MATERIALS AND METHODS

Study system

Gambusia affinis have simple husbandry and a wealth of data exist regarding their ecology and evolutionary biology (Meffe and Snelson, 1989; Scribner *et al.*, 1992; Smith and Belk, 2001; Langerhans and DeWitt, 2004). They have a diverse diet including zooplankton, detritus, epilithic films, insects, exuviae and, at some times and locations, snails and ostracods (Sokolov and Chvaliova, 1936; Harrington and Harrington, 1961; Garcia-Berthou, 1999). These resources typically vary spatially and temporally at multiple scales within and among generations for natural *G. affinis* populations. Given that these fish produce two to three generations annually (Haynes and Cashner, 1995), seasonal variation in resources is experienced as intergenerational variation.

Two populations of *G. affinis* in Brazos County, Texas, USA were chosen for this study based on differences in predator regime. Krenak Tap (30°36.6′N, 96°17.6′W) is a pond containing predatory sunfishes such as the largemouth bass, *Micropterus salmoides*, and green sunfish, *Lepomis cyanellus*. Hensel (30°37.5′N, 96°20.8′W) is a drainage canal with no piscivorous fish.

Rearing experiment

A common garden rearing experiment was conducted to estimate population effects and induced effects due to *how* (suction feeding vs. biting/scraping) and *where* (orientation) fish fed. We raised sibling groups of fish from the two populations in pure (invariant) food treatments, as is usual in plasticity studies. This pure environment approach simulated between-generation environmental variation. However, we also included an additional treatment where food orientation changed on a daily basis. The rotating treatment simulated fine-grained resource variation. We addressed spatial variation by using alternative populations wherein patterns of resource use likely differ due to differences in predation regime.

In March 2002, we collected gravid female *G. affinis* from each population and allowed them to deliver offspring in brood chambers mounted on either side of a large (284-litre) aquarium. The large tank was divided in half with a mesh panel that kept offspring from the two populations separate but allowed water exchange. Offspring delivered by females fell into the large tank volume where they remained until sufficient numbers were produced for the experiment. During brood production, hatchlings were fed twice daily on a rotation of live brine shrimp (*Artemia salina*; Order Decapoda), live microworms (*Panagrellus redivivus*; Order Nemata), live springtails (*Hypogastrura* sp.; Order Collembola) and spirulina flakes. Brooding females were fed brine shrimp and spirulina flakes. Over 21 days, 150 offspring were produced from 23 Krenek Tap females and 160 offspring were produced from 33 Hensel females.

We conducted the rearing experiment in a 2350-litre re-circulating system composed of twenty-four 75-litre aquaria, a 100-litre settling tank, a 300-litre biological filtration chamber and a sand filter through which water was circulated by a 1 horsepower pump. All tanks had a 5 mm mean particle diameter gravel substrate to a depth of 15 mm. We used a 12 : 12 h light : dark cycle and maintained water temperature at approximately 27°C. Each 75-litre tank was divided with a mesh panel, yielding a total of forty-eight 37.5-litre rearing sectors. Tanks were assigned to one of three food orientations (benthic, mid-water or water surface) or a rotation among orientations. One sector per tank was assigned to free prey and the other to attached food. Free prey items were chosen to reflect prey typical to each orientation: insects (springtails) at the surface, crustacean zooplankton (brine shrimp) in the water column, and nematodes (microworms) in the benthos. Free prey required fish to feed by suction, whereas attached food required a biting/scraping mode of foraging (C.B.R. and T.J.D., personal observations).

The experiment was planned as a 4 (3 static orientations + 1 rotating diet) × 2 (food types) × 2 (populations) factorial design of 16 treatments replicated three times. However, due to an error in tank assignment, there was one additional tank for some treatments (*bkp, bhl, mkp, mhl, rkp, rhl, tkp, thl*) and one fewer tank for others (*bkl, bhp, mkl, mhp, rkl, rhp, tkl, thp*), where the first letter indicates the orientation, the second letter is the population and the third letter is the type (live free vs. attached prepared foods) assigned to each tank. This error did not appear to produce bias. Phenotypic variance did not differ and all main effects were consistent between over- and under-represented tanks.

Six F_1 individuals were randomly assigned to each tank sector. Due to initial mortality, replacements were made after 11 days to bring the number in each tank to five. Mortality was unbiased by treatment ($P > 0.35$ in all cases). Nine days later, further replacements were made with non-experimental albino *G. affinis* to maintain sector densities while providing for easy distinction and subsequent exclusion from analysis. Mortality after the addition of albinos was low (6%) but was biased towards the surface feeding treatment ($F_{3,32} = 8.02$; $P < 0.001$) and the prepared food type ($F_{1,32} = 10.7$; $P = 0.002$). Thus, phenotypic differences across treatments with biased mortality could be due to selection, plasticity or both. To help discern this potential confluence of effects, we note here the effect strengths (and jackknifed ranges) for mortality bias of 0.43 (0.33–0.52) in the surface treatment and 0.25 (0.20–0.31) for the food type treatment. Jackknifed ranges were obtained by analysing the mortality data with one case excluded at a time until every case had been excluded once. In theory, this should set an upper bound on the extent of treatment effects on phenotype that could be due to selection. If treatment effects on phenotype exceed these values, then mortality bias cannot account for all of the effect and plasticity can be inferred to be operating.

Feeding took place twice daily as follows. Springtails were dropped onto the water surface where they floated until eaten. Brine shrimp were loaded into a 60 ml syringe and delivered through a dispensing tube into the middle of the water column. Microworms were delivered in a similar manner except into the substrate. The live-rotation treatment received one of the other three diets in an alternating pattern among days. Attached food was made by mixing gelatin and ground commercial food (Rangen Corp.) in hot water to produce a paste. This paste was extruded onto plastic discs with a template to standardize the amount and distribution of food on every disc. Discs were allowed to cool and then placed in tanks according to treatment. Surface discs were allowed to float, while mid-water and benthic discs were attached via suction cups to the side and bottom of tanks, respectively. Discs were replaced daily to ensure fresh food was available *ad libitum*.

After 60 days of rearing, fish were fixed in 10% formalin, rinsed with tap water overnight, and transferred to 70% ethanol for storage. Fish reaching sexual maturity (20 males, 1 female) or exhibiting gross shape anomalies by the conclusion of the experiments were excluded from analysis. This yielded a final sample size of 142 (for whole body morphology) and 148 (for pectoral fin analysis), or 3.2 ± 0.2 (mean ± standard error) fish per tank sector.

Morphometrics

Images of all specimens were captured from the lateral and dorsal perspectives. We then digitized eleven landmarks on the lateral images and six landmarks on the dorsal images (Fig. 1A,B). Bilateral landmarks in the dorsal perspective were reflected onto one another about the midline and averaged for analysis. However, for visualizations we retained the non-reflected coordinates. Pectoral fin images were captured by lowering fish, tail-first, through a gap between two horizontal glass plates and resting their pectoral fins flat against the glass. The camera was oriented perpendicular to the glass allowing for capture of both fin images simultaneously. Two landmarks were digitized on the pectoral fin insertions (Fig. 1C). The remaining points were treated as semilandmarks and located by projecting from the centroid of the fin at regular angles (as per Fig. 1C) onto the fin outline. Semilandmarks were allowed to slide along the outline to reduce the inflation of information (i.e. bending energies) associated with characterizing biological curves with deficient

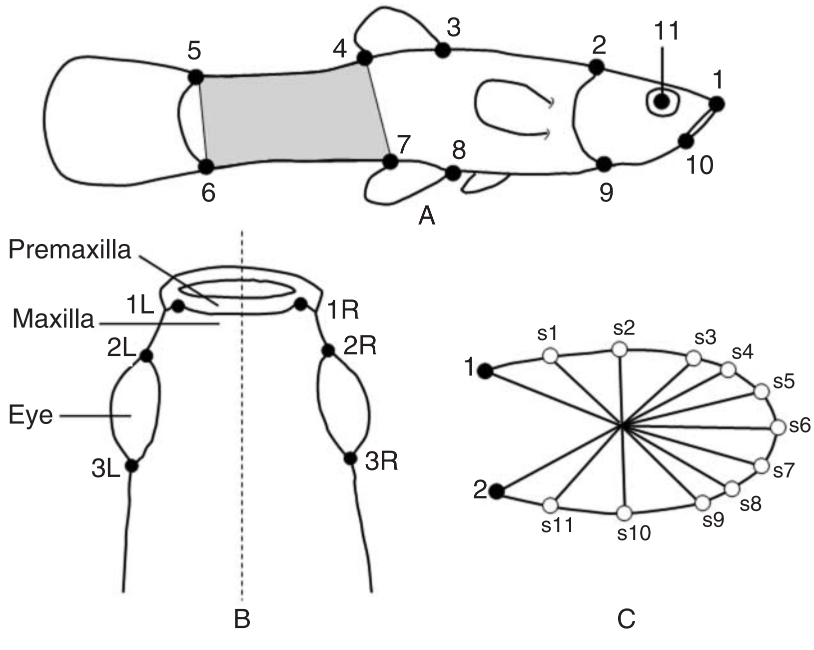

Fig. 1. Juvenile *G. affinis* with landmarks used for analysis. (A) Lateral view with shaded caudal peduncle. (B) Dorsal view of head. Bilateral landmarks (L, left; R, right) were reflected together about the midline (dashed line) and averaged for analysis. (C) Pectoral fin. Open symbols represent semilandmarks created by projecting from the fin centroid, along the radii shown, to fin margins. Semilandmarks (s1–s11) were treated as sliding landmarks following Bookstein (1991).

landmarks (Bookstein, 1991). These landmarks are considered 'deficient' because they were constrained to fall on our radii and so only had one dimension (distance along radii) in which their position could vary. We used both fins if they were intact by reflecting them into one aspect and averaging landmark coordinates ($n = 85$). If one fin was frayed, the intact fin was used (right only, $n = 40$; left only, $n = 23$). No difference in shape was noted between left and right fins (MANCOVA, approximate $F_{44,156} = 1.17$, $P = 0.24$). All landmarks were digitized at a resolution of 0.03 mm per pixel using MorphoSys software, Version 1.29 (Meecham, 1993).

Raw landmark coordinates were aligned by generalized least-squares superimposition. Shape variables (partial warps and uniform components) were calculated from aligned landmarks using tpsRelw (Rohlf, 2004). Such geometric morphometric techniques are powerful new tools that are increasingly common in studies of fish body shape (Cadrin, 2000; Langerhans *et al.*, 2004). Geometric methods differ from traditional methods because they retain information about the spatial covariation between landmarks, allowing the geometry of shape variation to be conserved throughout the analysis and reconstructed during visualization (Rohlf and Marcus, 1993).

Analysis

We tested for final size differences among treatment groups using analysis of variance (ANOVA). To analyse shape, we performed multivariate analyses of covariance (MANCOVA) for each aspect in which fish were imaged. Lateral, dorsal and pectoral landmark configurations yielded 18, 6 and 22 shape variables respectively. Shape data were tested for effects due to centroid size (covariate), food type (live prey or attached food), food orientation (surface, mid-water, benthic or rotation), population, all interactions between main effects, and tank nested within the type × orientation × population interaction (e.g. as in Table 1). Interactions between main effects and the covariate were also tested and removed from the model when not significant. Canonical axes from MANCOVA were visualized using TpsRegr (Rohlf, 2003a) and TpsSuper (Rohlf, 2003b). Visual inspection of thin plate spline transformation grids and examination of landmark correlations with canonical axes were used to determine the nature of morphological change due to each main effect. To test the specific hypothesis that the rotating treatment produced a phenotype intermediate between the three pure treatments (i.e. versus the alternative hypothesis of non-additive environmental effects), we conducted a multivariate contrast. This test compared the multivariate centroid for the rotation treatment against the mean of the other treatment centroids.

For the axis representing population differentiation, we wished to determine if the population effect could be related to both direct (predation *per se*) and indirect (trophic) effects of predation regime. Answering this question required comparison of our laboratory-reared fish with fish from a previous study (Langerhans *et al.*, 2004). For consistency with the other study, landmark 10 was removed from the lateral configurations of our laboratory-reared fish, and partial warps were regenerated for pooled data. Combining data sets allowed us to assign canonical scores for burst-swimming speed (predator escape) to our focal (laboratory-reared) fish. Thus, for each of our focal fish we had a population canonical score, canonical scores for feeding treatment effects, and canonical scores for predator escape, which is likely to reflect the direct effect of predators on population differentiation. We could then ask if the population differentiation we observed simultaneously reflected aspects of shape variation related to trophic and predation ecology. We addressed that question with a multiple regression. We regressed population effect scores with each of the four trophic axes and the single (direct) predator escape axis of shape variation. Significant relationships between our population effect and trophic or predator related axes would suggest population differentiation due to foraging, predation or interactions among them.

All statistical tests were conducted with JMP Version 4.04 software (SAS Institute Inc., Cary, NC). Polynomials were centred for interaction terms involving continuous independent variables. Bootstrapped ranges for multivariate effects sizes (partial η^2) were generated with SYSTAT Version 10.2 (SPSS Inc., Chicago, IL).

RESULTS

Final size

Final size did not differ for fish raised on alternative food types (i.e. live prey vs. attached foods: $F_{1,98} = 2.88$, $P = 0.1$), except indirectly through a weak interaction between food type and orientation ($F_{3,98} = 3.54$, $P = 0.02$; partial $\eta^2 = 0.095$). This interaction was due to elevated growth in the live/mid-water treatment, which indicated that brine shrimp was a better food than microworms, springtails or prepared foods. Size was also influenced by orientation ($F_{3,98} = 21.2$, $P < 0.0001$; partial $\eta^2 = 0.459$). Fish fed at the surface were 18% smaller on average at the end of the study, relative to fish raised in other treatments (Tukey HSD test, $\alpha = 0.05$). Final size also differed by population. Fish from the predator population (Krenek) were 9% larger at the end of the experiment than fish from the predator-free population (Hensel) ($F_{1,98} = 8.22$, $P = 0.005$; partial $\eta^2 = 0.077$).

Induced responses

Gambusia affinis body shape differed according to *how* (foraging mode) and *where* (orientation) fish fed (Table 1). Shape also differed between populations (Table 1). Multivariate allometry was evident but there was no evidence of differences in allometry by treatment (i.e. no heterogeneity of slopes for our covariate; $P \geq 0.4$ in all cases). Each of these effects is discussed in turn, below.

Food type

Fish fed free prey items or attached foods were morphologically distinct in all three aspects of shape (Table 1). The lateral, dorsal and pectoral fin models accounted for 44%, 21% and 45% of variation in their respective canonical axis scores. Fish raised on attached foods developed short, wide heads, lower snout positions, anteriorly shifted eyes and a sloping caudal peduncle compared with fish fed free prey (Fig. 2A,B; see Tables A1 and A2 in Appendix 1). Additionally, *G. affinis* fed attached food items developed longer, distally widened pectoral fins compared with fish raised on free prey (Fig. 2C; see Table A3 in Appendix 1). These differences likely result from phenotypic plasticity because the effect size observed for shape ($\eta^2 = 0.44$) exceeded the upper confidence interval of 0.31 calculated for mortality bias. That is, if biased mortality alone had produced bias in the final shape data, we would have expected an effect size less than 0.31.

Table 1. MANCOVA effects for *G. affinis* dorsal and lateral body shape and pectoral fin shape

Perspective	Effect	F	d.f.	P	Partial η^2
Lateral ($n = 142$)	Type (T)	3.44	18, 80	<0.001	0.44
	Orientation* (O)	1.85	54, 239.2	0.001	0.29
	Population (P)	1.90	18, 80	0.03	0.30
	O × P*	1.21	54, 239.2	0.2	0.21
	O × T*	1.04	54, 239.2	0.4	0.18
	P × T	1.13	18, 80	0.3	0.20
	O × P × T*	1.39	54, 239.2	0.05	0.24
	Tank (O × P × T)*	1.32	504, 1239.3	<0.001	0.30
	Centroid size	4.77	18, 80	<0.001	0.52
Dorsal ($n = 142$)	Type (T)	3.96	6, 92	0.001	0.21
	Orientation* (O)	1.34	18, 260.7	0.2	0.08
	Population (P)	1.08	6, 92	0.4	0.06
	O × P*	0.87	18, 260.7	0.6	0.05
	O × T*	1.22	18, 260.7	0.2	0.07
	P × T	1.18	6, 92	0.3	0.07
	O × P × T*	1.30	18, 260.7	0.2	0.08
	Tank (O × P × T)*	1.27	168, 549.6	0.02	0.28
	Centroid size	3.38	6, 92	0.005	0.18
Pectoral fin ($n = 148$)	Type (T)	3.00	22, 80	<0.001	0.46
	Orientation* (O)	1.04	66, 239.8	0.4	0.23
	Population (P)	0.87	22, 80	0.6	0.20
	O × P*	0.97	66, 239.8	0.5	0.21
	O × T*	0.91	66, 239.8	0.7	0.20
	P × T	1.86	22, 80	0.02	0.34
	O × P × T*	1.10	66, 239.8	0.3	0.23
	Tank (O × P × T)*	1.22	660, 1528.3	0.001	0.29
	Centroid size	2.76	22, 80	<0.001	0.43

Note: Effect sizes are given as partial η^2.* F was approximated using Wilks' λ.

Orientation

Food orientation affected lateral body shape (Table 1). The orientation effect accounted for a total of 29.2% of morphological variance captured by the three canonical axes from the MANCOVA on shape. Fish fed in the mid-water were distinguished from those in the surface and benthic orientations by the first (i.e. largest) canonical axis. The mid-water treatment induced shorter and deeper caudal peduncles, elongation of the dorsal and anal fin bases, terminal mouths and prostrate bodies (Fig. 3; see Table A1 in Appendix 1).

The second canonical axis separated fish fed at the surface from those fed in the other static orientations (Fig. 3). However, the effect size of mortality ($\eta^2 = 0.43$) was larger than that found for the orientation shape axis ($\eta^2 = 0.29$). A *post hoc* test of feeding orientations indicated that mortality in the surface treatment was higher than in the other treatments (Tukey HSD test, $\alpha = 0.05$). Thus, we do not have the ability to resolve whether the shape associated with surface feeding is due to plasticity or selection.

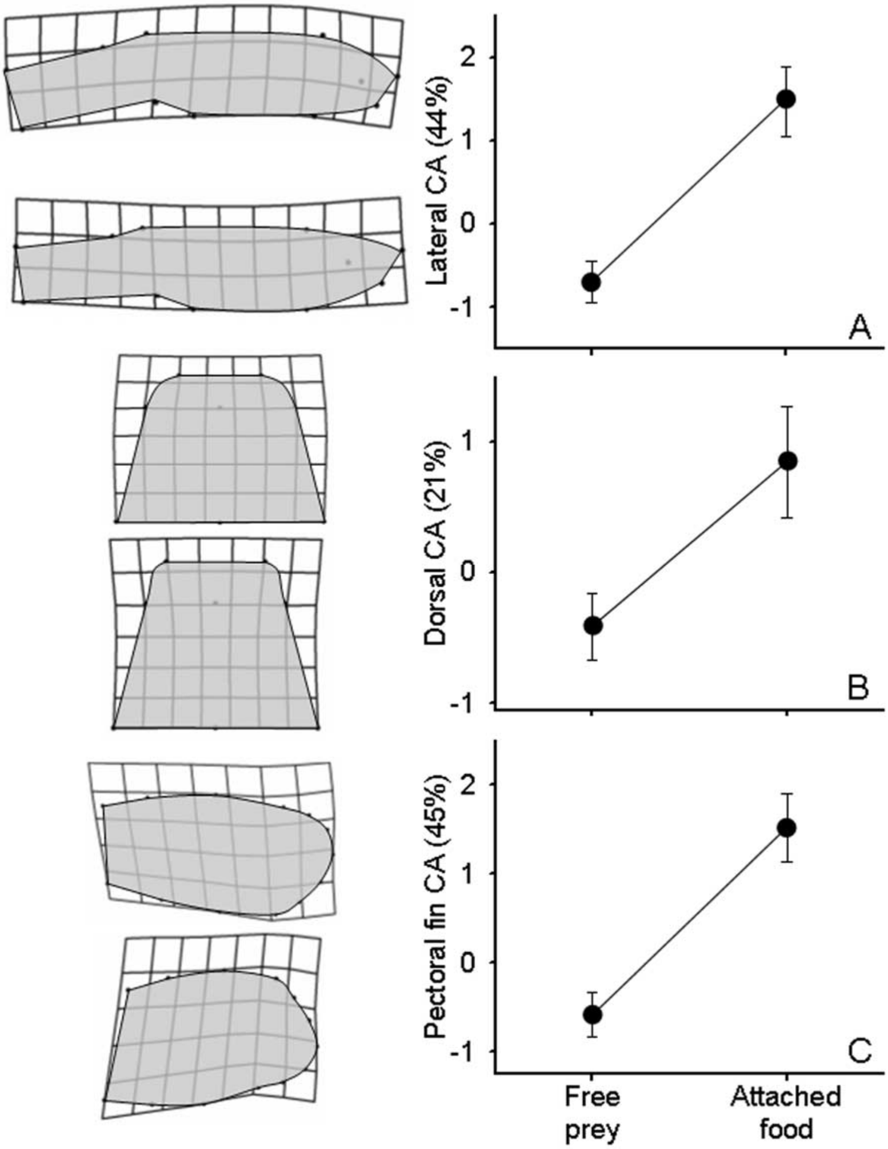

Fig. 2. Reaction norms and thin-plate spline visualizations of the food type effect in *G. affinis*. Transformation grids depict the observed range of shape effects along each shape axis (CA) in (A) lateral view, (B) dorsal view and (C) pectoral fins. Values in parentheses are the percent variation explained for each canonical axis. For animations of these morphological changes, see www.evolutionary-ecology.com/data/1785animations.html

Finally, the third food orientation axis captured aspects of shape variation peculiar to fish fed the rotating diet (CA3 in Fig. 3). The rotating treatment centroid did not differ from intermediacy in the overall multivariate space defined for the orientation effect ($F_{18,80} = 1.42$, $P = 0.147$; Fig. 3). Rotating diets induced shorter heads and caudal peduncles and

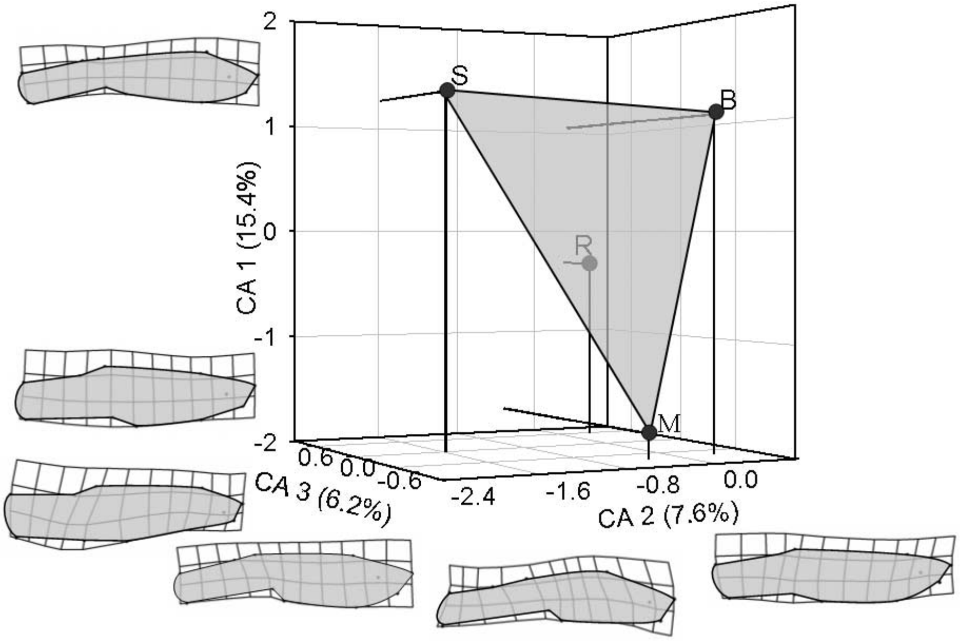

Fig. 3. Induced morphologies in *G. affinis* based on their feeding orientation. S = surface, M = mid-water, B = benthic, R = rotation among the three orientations. Transformation grids represent the observed range of canonical axes (CA) from our orientation treatment effect in MANCOVA. The vertical shape axis (CA1) distinguished fish fed in mid-water from those fed in the other static orientations. The horizontal axes describe differences between the surface and benthic feeding orientations (CA2) and differences in shape among fish fed a rotating diet from static orientations (CA3). Values in parentheses are the percent of shape variance accounted for by each canonical axis. These values, when summed, equal the total effect strength (partial η^2) for the orientation effect. Visualization of the major canonical axis (CA1) can be viewed online as an animation (see Fig. A4).

increasing caudal peduncle depth (Fig. 3; see Table A1 in Appendix 1). These results suggest that: (1) morphology induced by the fine-grained treatment was generally intermediate between the morphologies induced by the pure (invariant) treatments, and (2) fine-grained variation induced some distinctive aspects of shape.

Population differentiation

Gambusia affinis from the two populations differed in growth rate and shape, but not in the magnitude or nature of trophic plasticity. This suggests genetic differentiation in the traits, but not differentiation in levels of plasticity among traits [i.e. no gene × environment interaction, *sensu* Via and Lande (1985)]. Fish in the predator population grew 9% larger than fish from the predator-free population, indicating a possible (fixed) life-history shift. Lateral shape differences between the two populations (Table 1) accounted for 30% of variation in population canonical axis scores. The nature of shape difference involved an elongated peduncle, lower eye position, shorter head and less prostrate body in the predator population (Krenek Tap) relative to morphology in the predator-free population (Hensel) (see Table A1 in Appendix 1). The morphological difference in our laboratory-reared fish

Table 2. Partial correlations between population differentiation and aspects of resource- and predation-related shape effects

Effect	*F*	d.f.	*P*	Partial *r*
Food-related				
Food type CA score	0.01	1, 136	0.9	0.01
Orientation CA1 score	12.87	1, 136	<0.001	0.25
Orientation CA2 score	2.53	1, 136	0.1	0.14
Orientation CA3 score	22.38	1, 136	<0.001	0.40
Predation-related				
Burst speed score	14.91	1, 136	<0.001	0.35

Note: Effect sizes are given as partial *r*.

qualitatively resembled some of the trophic axes, as well as an axis in another study that related body shape and burst swimming speed (Langerhans and DeWitt, 2004; Langerhans *et al.*, 2004). To quantitatively compare the population differentiation axis with the trophic axes of the present study and the swimming performance (predator escape) axis from the other study, we combined the data sets and recalculated the MANCOVA (see details in Methods). This procedure resulted in similar significance structure and canonical axis compositions as the primary analysis. The multiple regression comparing the population effect to escape swimming and trophic morphology axes demonstrated both predator-like and trophic-like effects within the fixed population differences. Specifically, the population shape axis was associated with the swimming performance axis (predator related) and two of the three orientation axes (trophic related) but not the food type axis (Table 2).

DISCUSSION

Induced morphology and functional implications

We found extensive induced morphology among *G. affinis* from two populations when fed free prey or attached food in different orientations. The most pervasive influence on morphology was due to *how* (suction feeding vs. biting/scraping) fish fed, and will be discussed first. Capturing free prey generally involves picking, suction or ram feeding, whereas acquiring attached food requires biting or scraping (Barel, 1983; Webb, 1984; Winemiller, 1991). In this study, *G. affinis* used suction feeding to consume free prey. Fish hovered while locating prey items, manoeuvred into position, and sucked the prey from the surface, water column or benthos into their mouths. Narrow, long heads and distally narrow pectoral fins were evident in fish from the free prey treatment (Fig. 2; see animation in Figs. A1–A3 in Online Appendix). These morphological traits facilitate fine-scale targeting of suction streams steadied by manoeuvring (trimming) fins (Keast and Webb, 1966; Webb, 1982, 1984; Barel, 1983).

Gambusia affinis in our study acquired attached foods by biting/scraping. They did this by thrusting their bodies, jaws first, into the food while biting down. These fish developed wider heads, blunt snouts and long distally broad pectoral fins (Fig. 2; see animations in Figs. A1–A3 in Online Appendix). Blunt snouts are typically associated with scraping modes of foraging (Barel, 1983; Winemiller, 1991; Winemiller *et al.*, 1995; Kassam *et al.*, 2003) and distally broad pectoral fins indicate the ability to generate thrust over short distances (Keast and Webb, 1966; Barel, 1983; Webb, 1984). These findings for mosquitofish parallel those classically observed for taxa such as coral-*scraping* (i.e. versus *picking*) butterflyfishes (Motta, 1988) and epilithic-algae scraping cichlids (Barel, 1983). This parallel suggests the induced morphology within *G. affinis* may enhance feeding efficiency.

Morphology also varied among *G. affinis* fed in different orientations (Fig. 3). Fish fed in the mid-water developed terminal mouths, broad insertions for the dorsal and anal fins, prostrate bodies, shorter caudal peduncles, and increased body depth, especially between the dorsal and anal fins, compared with fish fed in other orientations (Fig. 3; see animation in Fig. A4 in Online Appendix). Deep bodies are typical for fish species that hover in the water column and pick at free prey items (Keast and Webb, 1966; Barel, 1983; Motta, 1988). However, this trend is not consistent with two other published cases of intraspecific variation associated with habitat use. Schluter (1993) found sticklebacks (*Gasterosteus* spp.) to have streamlined, slender bodies in open water habitats compared with sticklebacks from benthic habitats. Similar results have been found in Eurasian perch (*Perca fluviatilis*) (Svanback and Eklov, 2002). Thus, patterns of intraspecific morphological divergence across habitat gradients, which presumably are due to alternative resource use, can vary. This lack of consistency suggests the need for further experimentation to separate causal and correlative effects on morphology.

In addition to comparing plasticity in this species with ecomorphological results in other taxa, we wished to address, in at least a preliminary manner, two relatively unexplored topics associated with phenotypic plasticity: (1) responses of plasticity to fine-grained environmental variation, and (2) the intersection of resource-based plasticity with other ecological domains.

Fine-grained environmental variation

Environments often vary on time scales much less than the generation time of an organism. Such fine-grained variation can reduce the efficacy of phenotypic plasticity because phenotypic responses may lag behind the pace of environmental change (Bradshaw, 1965; Levins, 1968; Padilla and Adolph, 1996). Thus, plasticity is seen mainly as a solution for coarse-grained environmental variation, with three exceptions. The first exception comes when variation is predictable in advance based on indirect cues, allowing for phenotypic responses before the environment actually changes. For example, many organisms condition development on photoperiod cues that indicate impending environmental shifts [e.g. cyclomorphosis in *Daphnia* (Dodson, 1989)]. The second exception is for modular or indeterminately growing organisms. Such organisms can continually adapt at least part of their morphology to prevailing conditions (Bradshaw, 1965; Gosler, 1986; Meyer, 1987; Harvell, 1992). The third case is behaviour, which can be changed rapidly, but is excluded by some from the realm of plasticity (Silvertown, 1998; Sih, 2004).

An evolutionary response to fine-grained environmental variation has been explored in at least one case (Kassen and Bell, 1998). These authors experimentally examined the evolution of generalists and specialists in both coarse- and fine-grained environments for a unicellular chlorophyte. They hypothesized that specialists evolve in constant environments and, depending on the temporal scale of environmental variation, at least two types of generalist evolve. Kassen and Bell (1998) defined environmental variation occurring once every few generations as coarse-grained, while variation within a generation was considered to be fine-grained. They further explained that a ‘plastic generalist’ should evolve in response to coarse-grained variation and a ‘versatile generalist’ should evolve in response to fine-grained variation. We substitute the word ‘dynamic’ in place of ‘versatile’ because we feel it is more descriptive of development, rather than the functional effect of developmental plasticity. Kassen and Bell (1998) produced both specialists and generalists in constant and variable environments, respectively. However, there was no evidence for the evolution of a ‘versatile’ (dynamic) or ‘plastic’ generalist in response to any grain of environmental variation. In the present study, we examined whether fine-grained environmental variation could produce intermediate (generalist) morphology in a vertebrate, *G. affinis*.

Our thesis in this section is that plasticity can produce generalist phenotypes in addition to its classically understood role of producing relatively specialized morphologies across coarse-grained environmental gradients. Under fine-grained environmental fluctuation (i.e. daily environmental switching), plasticity may produce phenotypes reflecting a weighted sum of environments experienced – a dynamic generalist. Such a summation may or may not weight all environments equally. In our case, there was no evidence of unequal weighting of environmental effects. Rather, for our major axes of shape variation, an additive intermediate morphology was produced. Such a strategy (weighted generalist phenotypes through plasticity) is more flexible than a canalized generalist strategy because it allows organisms to accommodate to prevailing conditions instead of having a fixed developmental programme. Thus, plasticity may often be superior to canalized generalization for dealing with fine-grained environmental variation.

In addition to producing an intermediate phenotype under fine-grained variation, both canalized generalists and plastic generalists could also evolve to produce distinctive phenotypic elements, specifically for dealing with fluctuating environmental regimes. In our case, the third shape axis of the food orientation effect revealed distinctive aspects of morphology associated with the rotating (fine-grained variation) treatment (Fig. 3). This effect was small in the present study, but suggests the potential importance of the idea for other traits, and in other organisms, where either plastic or canalized generalist strategies might exist. This could overturn the usual jack-of-all-trades metaphor when thinking about generalists. Generalists are usually conceived as possessing the same tools as specialists, but to lesser extent. However, the generalist jack-of-all-trades may have unique tools.

In the present system, as in most, we do not know the precise nature of environmental variation in the focal populations. We therefore cannot comment on the adaptive value of specific morphologies induced by fine-grained environmental fluctuation in this study. Recent work is focusing on documenting the precise patterns of environmental variation in natural populations of annual herbs (Huber *et al.*, 2004). We suggest the parallel importance of including fine-grained variation as treatments in plasticity studies. Together these efforts should yield increased insight regarding the evolution of plasticity in complex natural systems.

Population differentiation

Gambusia affinis from the two populations differed in growth rate and shape, but not in the magnitude or nature of trophic plasticity. This suggests genetic differentiation in the traits, but not differentiation in levels of plasticity among traits [i.e. no gene × environment interaction, *sensu* Via and Lande (1985)]. Fish originating from the predator population grew

9% larger than fish from the predator-free population. This may indicate a possible life-history shift in the predator population. Faster growth in predator populations has been observed in several livebearers (e.g. Reznick, 1996; Johnson, 2001) and is a classic expectation of life-history theory (Roff, 1991; Kawecki and Stearns, 1993). However, our primary focus in this study was fish shape.

Fish from the predator population developed some aspects of morphology (e.g. long caudal peduncle, lowered eyes) similar to those found in another study that considered more populations (Langerhans *et al.*, 2004). Other aspects of morphology (e.g. short heads) appeared to be related to resource acquisition. Pooling the two data sets allowed us to assign a predation-related (burst speed) score to our laboratory-reared fish. This enabled us to address preliminarily the indirect effects of predators on prey morphology through forced habitat shifts.

Predator-induced shifts in resource base are common (Abrams, 1995; Relyea, 2000; Bernot and Turner, 2001). In the present study, resources did not seem to differ between populations (i.e. food present in all orientations), but habitat use differed. *Gambusia affinis*, in populations with predators, are forced to forage in fringe habitats that potentially have a different resource base (R.B. Langerhans and T.J. DeWitt, personal observation). Thus, we expected that population differentiation could reflect both the direct effects of predators (e.g. morphology to increase escape swimming) and indirect effects of predators due to habitat shifts (e.g. trophic morphology).

Our results indicated that the population effect was related to three of the four trophic axes and to the predation (burst speed) axis (Table 2). This supports our hypothesis, in at least a preliminary manner, that aspects of population differentiation may be related to both direct and indirect impacts of predators. Predator escape morphologies (long peduncles, lowered eyes), *sensu* Langherhans *et al.* (2004), and morphologies associated with feeding in orientations other than mid-water (shorter medial fin insertions, arched bodies) were evident in our predator population. This result is consistent with the hypothesis that predators (e.g. *Micropterus salmoides*) exclude *G. affinis* from feeding in the mid-water, forcing them to feed on alternative food items found in fringe habitats. This idea is the subject of ongoing testing and directed experimental work.

We sought to create a well-controlled environment where we could test for effects on morphology due to coarse- and fine-grained environmental variation, population differentiation, and take a preliminary look at the possibility of indirect effects of predators on trophic morphology. The morphological variation observed in many ways resembled general morphological paradigms across species and may represent adaptive variation. Plasticity is classically expected to produce adaptive solutions to coarse-grained environmental fluctuation. We argued that plasticity may also be a good strategy for dealing with fine-grained environmental variation. Fine-grained variation is likely for most environmental gradients but is only rarely included in plasticity studies. Plasticity also has the potential to act in complex indirect ways. For example, since predators alter prey habitat choice, the next expectation is that prey morphology would also be affected. Our study revealed that population differentiation potentially was related to both direct (escape swimming) and indirect (trophic) effects of predation. However, the interaction between predation and trophic morphology requires subsequent experimentation to firmly establish the link between these two ecological domains. Results of this experiment suggest the utility of including multiple scales and multiple ecological domains in future studies of phenotypic plasticity.

ACKNOWLEDGEMENTS

We thank R. Brian Langerhans, Kirk Winemiller, Craig Layman, David Hoeinghaus and two anonymous reviewers for offering suggestions on our manuscript. Heather Prestridge and Clint Wilson provided technical assistance during the experiment. We thank Dean Adams for helpful discussions on sliding landmarks. This work was supported by a Sustainable Coastal Margins Program Fellowship to C.B.R. and partly by NSF grant #DEB-9908528 to T.J.D. This is a contribution from the Faculty of Ecology and Evolutionary Biology at Texas A&M University.

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APPENDIX 1

Table A1. Pearson correlations between the superimposed lateral landmark coordinates and the three main effects: food type (free prey, attached food), orientation (surface, mid-water, benthic, rotating) and population of origin (variables exhibiting a correlation ≥ | 0.2 | are in **bold**)

		Orientation			
Landmark	Food type CA	CA 1	CA 2	CA 3	Population CA
1X	**−0.456**	0.131	−0.039	**−0.284**	−0.123
1Y	**−0.579**	**−0.323**	**0.238**	0.060	**−0.354**
2X	**0.311**	0.133	**0.423**	0.193	**0.345**
2Y	**0.482**	**0.268**	**−0.457**	−0.014	−0.068
3X	0.191	**−0.231**	−0.115	**0.198**	**−0.419**
3Y	**0.390**	**−0.503**	0.067	0.014	−0.039
4X	−0.180	0.069	**0.296**	0.127	**−0.299**
4Y	0.135	−0.144	**0.229**	−0.142	−0.181
5X	−0.161	−0.046	−0.107	−0.008	**−0.352**
5Y	**−0.427**	**−0.358**	0.039	**0.380**	**−0.426**
6X	0.085	**0.233**	**0.334**	−0.027	0.179
6Y	**−0.529**	−0.053	0.064	−0.035	0.040
7X	−0.020	−0.147	**−0.438**	**−0.360**	**0.338**
7Y	**0.341**	**0.533**	**−0.321**	**−0.530**	**0.557**
8X	0.003	**−0.306**	**−0.297**	0.169	**0.327**
8Y	**0.345**	**0.534**	−0.183	−0.178	0.540
9X	0.145	0.010	−0.008	−0.077	−0.082
9Y	**0.507**	0.013	−0.163	**0.454**	−0.073
10X	**−0.457**	0.120	0.036	−0.096	−0.128
10Y	**−0.349**	**−0.195**	**0.448**	**0.224**	−0.040
11X	**0.479**	0.111	**−0.229**	**0.262**	**−0.253**
11Y	**−0.232**	−0.012	0.099	−0.073	**−0.271**

Table A2. Pearson correlations between superimposed dorsal landmark coordinates and the food type (free prey vs. attached food) (correlations ≥ | 0.2 | are in **bold**)

Landmark	Food type CA
1X	**−0.327**
1Y	**−0.426**
2X	**0.504**
2Y	0.095
3X	**0.379**
3Y	**0.367**

Table A3. Pearson correlations between superimposed pectoral fin landmark coordinates and the food type effect (correlations ≥ | 0.2 | are in **bold**)

Landmark	*r*
1X	**−0.448**
1Y	**−0.219**
s 1X	**−0.415**
s 1Y	**−0.329**
s 2X	**−0.253**
s 2Y	**−0.331**
s 3X	0.026
s 3Y	**−0.349**
s 4X	0.172
s 4Y	−0.119
s 5X	0.186
s 5Y	0.084
s 6X	0.088
s 6Y	0.166
s 7X	0.103
s 7Y	0.063
s 8X	0.175
s 8Y	−0.060
s 9X	**0.260**
s 9Y	−0.147
s 10X	**0.214**
s 10Y	0.141
s 11X	0.111
s 11Y	**0.417**
2X	0.006
2Y	**0.608**