Shared and unique features of predator-associated morphological divergence in three livebearing fishes

R. Brian Langerhans
Craig A. Layman
A. Mona Shokrollahi
Thomas J. DeWitt

Department of Wildlife and Fisheries Sciences
Texas A&M University, 2258 TAMU
College Station, TX 77843-2258, USA
Fax 979-845-4096

Contact information:
R. B. Langerhans: phone: 979-845-7522; e-mail: brianl@tamu.edu
C. A. Layman: phone: 979-862-1279; e-mail: cal1634@yahoo.com
A. M. Shokrollahi: e-mail: mona_shok@hotmail.com
T. J. DeWitt: phone: 979-458-1684; e-mail: tdewitt@tamu.edu

Keywords: divergent natural selection, fitness trade-offs, geometric morphometrics, phenotypic plasticity, population differentiation, predation

Running Head: Morphological divergence in livebearers

ABSTRACT: Divergent natural selection (fitness trade-offs) between environments often drives intraspecific diversification. When multiple species face a common environmental gradient their patterns of divergence might exhibit both shared and unique elements. We investigated shared and unique elements of diversification for three species of livebearing fishes (Poeciliidae) across a predator gradient (piscivorous fish vs. no piscivorous fish). All species (*Gambuia affinis* from the United States, *G. hubbsi* from the Bahamas and *P. reticulata* from Trinidad) exhibited a posterior shift in body allocation and more elongate bodies in predator populations. This morphological shift applied not only across species, but across age/gender classes, particular predator species and gross habitat types. Each species also had unique features of divergence, which may reflect peculiarities unique to their natal environments. We hypothesize much of the predator-associated morphology enhances fitness in the presence of predators but decreases fitness in the absence of predators. Preliminary evidence supports this hypothesis. We additionally found gonopodium size divergence in both *Gambusia* species that was opposite a trend previously found in *P. reticulata*. Partitioning phenotypic variation for multiple species into shared and unique responses across environmental gradients is a useful tool for elucidating general and localized mechanisms of diversification.

Divergent natural selection (DNS) is a powerful mechanism that generates and maintains phenotypic diversity (Rice and Hostert 1993; Orr and Smith 1998; Schluter 2000*b*). DNS is created by functional trade-offs between alternative environments (Robinson and Wilson 1994; Robinson et al. 1996; Schluter 2000*b*). Depending on how environments vary (e.g. fine- or coarse-grained variation; sensu Levins 1968), DNS can lead to evolution of either canalized genetic differences between populations or phenotypic plasticity (West-Eberhard 1989; Robinson and Wilson 1994; Orr and Smith 1998; Schluter 2000*b*). DNS results in population differentiation through either of these mechanisms. That is, populations may differ from each other because of fixed genetic differences or because each is similarly plastic but their current environments differ. Environmental differences that typically produce population divergence include predation, competition, or abiotic stresses (Magurran and May 1999; Schluter 2000*b*).

Consider the case where the sign of selection on focal traits differs across environments. Consider also that several species are evolving adaptive solutions in these environments. Some elements of evolutionary response will be similar across species. For example, species A and B may largely respond in a similar manner. Yet other aspects of response to DNS will be species-specific (A responds one way; B responds differently). In theory, after accounting for gross differences between species, the unique and shared elements of response to DNS can be identified. This approach allows one to integrate the study of phenotypic variation across selective environments, populations and species.

Empirical studies of diversification in vertebrates has focused on resource competition and niche partitioning (Robinson and Wilson 1994; Van Valkenburgh and Wayne 1994; Smith and Skulason 1996; Schluter 2000*a*). However, predation is among the most important factors structuring natural populations (Sih et al. 1985; Kerfoot and Sih 1987; Jackson et al. 2001).

Predators should not only change the distribution and abundance of species (via lethal and behavioral impacts on prey), but should also produce diversification—changes in the distribution and abundance of phenotypes within species (Endler 1995; DeWitt et al. 2000; Trussell 2000; Van Buskirk and Schmidt 2000).

Although predation is often thought responsible for the evolution of inducible defenses among invertebrates (Harvell 1986; Havel 1987; Tollrian and Harvell 1999), studies investigating the importance of predation in vertebrate diversification are less common. Most studies of vertebrate evolutionary responses to predation center on life history changes (e.g. Skelly and Werner 1990; Rodd and Reznick 1997; Belk 1998; Johnson and Belk 2001; Reznick et al. 2001). Investigation of morphological divergence between alternative predator environments in vertebrates is less common (Brönmark and Miner 1992; Van Buskirk et al. 1997; Walker 1997; Lardner 2000; Relyea 2001).

In this study, we examined body shape divergence in three species of livebearing fishes across alternative predator regimes. Furthermore we evaluate trends within and between species to identify both unique and shared aspects of diversification among prey species.

Study System

Species of the livebearing family Poeciliidae are ideal models for evolutionary studies, and predator-driven diversification is well documented for some poeciliids (e.g. Meffe and Snelson 1989*b*; Endler 1995; Reznick 1996; Reznick et al. 1997). These fish are small-bodied, mature rapidly, and inhabit a wide variety of environments (e.g. high and low predation). Differences in life-history characteristics and demography in relation to predation from piscivorous fishes have been especially well described for several species in this family (Krumholz 1963; Sohn 1977; Reznick 1989; Rodd and Reznick 1997; Downhower et al. 2000; Johnson and Belk 2001). These differences reflect fitness trade-offs between high- and low-predation environments. The genetic basis and role of phenotypic plasticity in several life history traits of poeciliids have also been evaluated (reviewed in Meffe and Snelson 1989*b*; Meffe et al. 1995; Reznick and Bryga 1996; Rodd et al. 1997). However, few studies have explored morphological diversification in poeciliids other than color (e.g. Endler 1978; Endler 1980, 1983; Winemiller et al. 1990) or trophic plasticity (Robinson and Wilson 1995). Research on body shape differences in poeciliid populations under alternative predation regimes has only just begun (this study; C.K. Ghalambor, D.N. Reznick and J.A. Walker unpublished data). This study examines morphological variation associated with intensity of predation by fish among populations of three poeciliid fish (*Gambusia affinis*, *G. hubbsi*, *Poecilia reticulata*).

Across the southeast United States, populations of Western mosquitofish (*Gambusia affinis*) are found in habitats ranging from drainage ditches to lakes and rivers, all of which have widely varying suites of aquatic predators (Meffe and Snelson 1989*a*). We compared morphologies of *G. affinis* from populations that differed in predator regime in Texas, USA (three populations with abundant fish predators and three without piscivorous fish). We additionally analyzed two populations of the congeneric *G. hubbsi* that differed in predator regime on Andros Island, Bahamas. *G. hubbsi* inhabit many connected and isolated waterways on Andros Island that differ in abundance of fish predators. To further evaluate the generality of morphological divergence, we consulted a historical dataset of four populations (two high-predation and two low-predation) of the Trinidadian guppy (*Poecilia reticulata*). *P. reticulata* represents a different tribe of the family (Poeciliini vs. Gambusiini; Rosen and Bailey 1963) and inhabits small streams which vary in predator regime in northeastern Venezuela and Trinidad (Rosen and Bailey 1963; Seghers 1974; Endler 1978). Life history and body color divergence in *P. reticulata* have been studied extensively (reviewed in Endler 1995; Houde 1997). For each fish species we examined how morphologies differed across predator environments in manners that may reflect adaptive solutions to alternative environments.

Methods

Gambusia affinis

Populations—

We collected *Gambusia affinis* from six populations in Brazos County, Texas, USA. To test for morphological variation potentially caused by piscivorous fish, sites were chosen *a priori* so that they differed primarily in predator regime. Three populations contained fish predators (Krenek Tap, 30º36.6'N, 96º17.6'W; Riverside Campus A, 30º38.1'N, 96º27.9'W; University Oaks, 30º37.2'N, 96º18.8'W) and three lacked piscivorous fishes (Hensel, 30º37.5'N, 96º20.8'W; Riverside Campus B, 30º38.0'N, 96º28.4'W; Autumn Circle, 30º38.4'N, 96º19.7'W). *G. affinis* are prey for a diverse array of predators, but one of their greatest threats is from piscivorous fish (Meffe and Snelson 1989*a*). Throughout this paper, populations of *Gambusia* are termed predator and predator-free in relation to piscivorous fish, although other predators may be present (e.g. invertebrates, turtles, birds). We specifically sought sites as similar as possible in environmental variables other than predator regime (e.g. aquatic vegetation, velocity, turbidity), however two general categories of aquatic environments, ponds and ditches, were represented within each predator regime. This abiotic habitat parameter (pond/ditch) was used in our analysis to statistically control for possible habitat/predator covariation and ensure predator effects were independent of this abiotic parameter.

We surveyed populations for predators using seines, cast nets and dip nets. Common predators were native sunfishes (family Centrarchidae), including largemouth bass (*Micropterus salmoides*), green sunfish (*Lepomis cyanellus*), longear sunfish (*Lepomis megalotis*), and bluegill sunfish (*Lepomis macrochirus*). We collected *G. affinis* using seines and dip nets and immediately transferred them to a laboratory at Texas A&M University. In the laboratory, live fish were separated into three age/gender classes: males, females and juveniles. Juveniles were defined as individuals under 20 mm total length that could not be sexed. We obtained morphometric data from 410 individuals with at least 21 individuals of each age/gender class from each population.

Morphometrics—

Images of *Gambusia affinis* were captured with a video imaging system and measured using MorphoSys (V. 1.29) software (Meecham 1993). Live fish were placed into a glass cage (30 mm high x 40 mm long x 5 mm wide) in front of a video camera equipped with a telecentric lens (Computar model TEC-M55) to obtain lateral images. The narrow cage prevented turning and restricted maneuverability, thus maintaining a constant angle of view. Video was captured at a resolution of 0.05 mm / pixel. We captured images of live fish to enable subsequent swimming performance analyses with these fish. We digitized body outlines and 12 landmarks for each fish using MorphoSys software (fig. 1a). Landmarks 1-10 were digitized directly from images and landmarks 11-12 were computed geometrically from coordinates of digitized points. From the outlines and landmarks we obtained both traditional and geometric morphometric data from the *G. affinis* images.

Traditional morphometrics evaluates shape variation by analyzing linear distances, angles, and meristic counts, often using multivariate statistics (Rohlf and Marcus 1993). Within the traditional morphometric approach, we calculated 13 measurements from our 12 landmarks (fig. 1a). These measurements included four lateral lengths (snout, caudal peduncle, caudal fin and standard lengths), three body depths (head, midbody and caudal peduncle depths), five areas (logarithm of dorsal fin, caudal fin, anal fin, caudal box and total area) and one ratio (standard length / middle-body depth). Total area was calculated as the area within the entire body outline, including fins. We obtained fin areas by isolating the fin from the body outline with a line connecting the fin insertion points. Caudal box area was defined as the region enclosed by landmarks 4-5-6-7 (shaded region in fig. 1a). We created the caudal box concept to isolate a functionally important portion of the fish body that determines fast-start (predator-escape) swimming performance (Webb 1984). Aspect ratio was taken as the ratio of standard length (LM 1-11) to middle-body depth (length of line halfway along and perpendicular to line 1-11). This measurement provides a gross estimate of overall shape, where higher values represent more elongate bodies and lower values represent deeper-bodied individuals. All morphometric data were highly consistent within individuals ($P < 0.001$), including fin areas, which are typically the most variable measures (average repeatability for male fins: $r_I = 0.88$; for female fins: $r_I = 0.90$).

We used landmarks 1-10 in our geometric morphometric analyses. Geometric morphometrics is a powerful new tool for analyzing shape variation (Rohlf and Marcus 1993) and has recently been applied to fish shape (e.g. Walker 1997; Walker and Bell 2000; Douglas et al. 2001). The distinguishing feature of geometric morphometrics is that it retains information on spatial covariation among landmarks; traditional approaches use one-dimensional distances without consideration of where a given measurement is taken relative to others.

We used tpsRegr software (Rohlf 2000*a*) to obtain superimposed landmark coordinates for analyses. TpsRegr rotates, translates, and scales landmark coordinates into alignment via generalized least squares superimposition and re-expresses coordinates as bending energies using thin-plate-splines (Bookstein 1991). Superimposed landmark configurations for all individuals within age/gender classes were used to calculate affine and nonaffine shape components (i.e. partial warps) using tpsRegr. Affine components of shape variation describe uniform spatial covariation in the x and y planes. Affine components are described by two orthogonal parameters, uniform X (shearing) and uniform Y (stretching). Shearing of a structure shifts all points parallel to one another, such as when a square is sheared into a parallelogram. Stretching also shifts points parallel with each other, but without shearing, such as when a square is stretched into a rectangle. Nonaffine transformations describe inhomogeneous changes (local deformations) and are summarized by geometrically orthogonal components (subsequent partial warps, PW).

Statistical Analyses—

All statistical analyses were conducted using JMP software (Version 4.04, SAS Institute Inc., Cary, NC). Males, females and juveniles were analyzed separately. For each age/gender class, we performed three nested multivariate analyses of covariance (MANCOVA) to evaluate morphological differences among populations of *G. affinis*. The three analyses represented different morphometric approaches: 1) traditional morphometrics, 2) geometric morphometrics, and 3) a hybrid of both techniques. Shape data were tested for effects attributable to size (covariate), predator regime, and population nested within predator regime. So our "standard" nested MANCOVA is:

Shape = Constant + Size + Predator regime + Population(Predator regime)

For the traditional morphometrics model, our 12 measurements served as shape variables, whereas our 16 partial warps were dependent variables in the geometric morphometric model. To use the powerful resolution of geometric morphometric data while not losing unique aspects of shape captured by the area variables, we performed a hybrid analysis. The hybrid analysis combined geometric morphometric data with four fish areas (dorsal fin, caudal fin, anal fin, caudal box). Size for the traditional model was the logarithm of total area. For geometric and hybrid models centroid size served as the size variable. The nature of morphological divergence was assessed by examining loadings of the predator regime canonical axis.

We performed an additional test to control for the possibility of covariance between predator regime and habitat type (pond/ditch). To address this, we conducted a two-step analysis. We first performed a MANOVA testing effects of pond/ditch on body shape data (using hybrid data) and saved residuals from this model. We then used these "habitat-free" residuals as shape variables in a nested MANCOVA equivalent to that described above. This technique evaluates morphological divergence between predator regimes after controlling for a putative pond/ditch effect.

To test for an effect seen in another poeciliid fish (*P. reticulata*, Kelly et al. 2000), we contrasted gonopodium (sperm intromittent organ) size between predator regimes using nested analysis of covariance (ANCOVA). The logarithm of gonopodium area was tested for effects attributable to centroid size (covariate), predator regime, and population nested within predator regime.

As in previous studies of this nature (e.g. Reznick and Endler 1982; Reznick 1989; Kelly et al. 2000), populations were treated as fixed effects since differences in population means, within predator regime, were of interest (Bennington and Thayne 1994; Sokal and Rohlf 1995, pp. 196-203). *F* ratios used type-III sums of squares. For all MANCOVA and ANCOVA models, homogeneity of slopes was verified before inclusion of covariates.

Gambusia hubbsi

Populations—

We collected *Gambusia hubbsi* from two populations on Andros Island, Bahamas. Populations were chosen *a priori* so that they differed in predator regime: one population contained piscivorous fishes (24º47.5'N, 77º48.6'W) and one did not (24º55.1'N, 77º57.8'W). Populations were sampled using seines, cast nets and dip nets. We collected a total of 45 females and 23 males of *G. hubbsi*. Primary predators of *G. hubbsi* in the predator site were great barracuda (*Sphyraena barracuda*), redfin needlefish (*Strongylura notata*), Atlantic needlefish (*Strongylura marina*), tarpon (*Megalops atlanticus*) and spinycheek sleeper (*Eleotris pisonis*). Detailed descriptions of fish communities on Andros Island are presented elsewhere (Downhower et al. 2000; Nagelkerken et al. 2001; Layman and Silliman in press). Fish were frozen and brought to the laboratory at Texas A&M University.

Morphometrics—

We captured images of *G. hubbsi* using the same video camera and lens as for *G. affinis*. Specimens were thawed immediately prior to image capture and placed on their side to obtain lateral images. Landmarks were digitized as described above. *G. hubbsi* were not alive, so fin areas comparable to *G. affinis* could not be obtained. No juveniles were collected from the predator population, so analyses were conducted only for adult males and females.

Statistical Analyses—

Separate MANCOVAs were conducted for males and females of *G. hubbsi* using partial warps as dependent variables to test for morphological effects due to size (covariate) and predator regime. After assessing species-specific divergence (for *G. hubbsi* alone) we combined data for *G. affinis* and *G. hubbsi* to evaluate morphological divergence in both species simultaneously. With the combined dataset, we performed our standard nested MANCOVA separately for males and females. This model placed both species on the same morphological axis so we could assess similarity between species in predator-associated diversification.

We additionally tested for differences in gonopodium size in male *G. hubbsi* between predator regimes using ANCOVA. The logarithm of gonopodium area was tested for effects attributable to centroid size (covariate) and predator regime. We then combined *G. affinis* and *G. hubbsi* gonopodia data and analyzed the two species together in an ANCOVA as described for *G. affinis* to evaluate similarity between species in gonopodia divergence across predator regimes.

Poecilia reticulata

Populations—

We examined published photographs of *P. reticulata* males from four populations in Trinidad that differed in predator regime (Endler 1978). Two populations contained the major guppy predator *Crenicichla alta* as well as other piscivorous fish (*Astayanax bimaculatus*, *Hemibrycon dentatum*) (high predation; Aripo 6, N=15; Ceniza 1, N=15). The two other populations contained only the meager predator *Aequidens pulcher* (low predation; Aripo 9, N=18, Quare 4, N=19). Diet studies indicate piscivory by *A. pulcher* is extremely rare (i.e. about 1 instance per 1000 fish, Seghers 1973; Endler 1978).

Morphometrics—

To analyze the morphology of *P. reticulata* we digitized scanned images of males (0.08 mm / pixel). The photographs provided clear body outlines but fin areas were difficult to discern. So we excluded fins and obtained only geometric morphometric data for guppies. A medial axis technique was used to capture shape information (fig. 1b). We digitized three landmarks directly (LM 1, 10, 11 in fig. 1b) and computed eight points geometrically from the medial axis (line 1-12 in fig. 1b). These eight landmarks were calculated as pairs of points on the body outline perpendicular to the medial axis at 20%, 40%, 60% and 80% distance from LM 1.

Statistical Analyses—

We performed our standard MANCOVA using partial warps as shape data to examine morphological divergence between predator regimes. To provide comparable data for the other species we conducted the same medial axis technique with males of *G. affinis* and *G. hubbsi*. We then performed a collective MANCOVA with data from all three species.

To obtain a more focused perspective on how *responses* of the species exhibited unique and shared characteristics of morphological divergence, we performed a two-step analysis. First, we performed a MANOVA that tested for species effects on shape (PWs) and saved residuals from this model. We then performed our standard MANCOVA using residual shape data as the dependent variables. This allowed us to examine the response of each species independent of gross-level shape differences between species.

We additionally assessed magnitudes of shared and unique responses to predators by conducting a discriminant function analysis (DFA). This DFA simply used partial warps as the dependent variables and predator regime as an independent variable. This simple DFA is conservative in that it classified individuals into predator regime without controlling for variation due to population or species. The reason we applied this approach is that DFA provides intuitive metrics regarding the magnitude of shared responses (e.g. percent of fish correctly classified into predation regime).

Results

Our study revealed *Gambusia affinis* males, females and juveniles exhibit significant morphological differences between predator and predator-free populations (Table 1). Shape varied with size for each age/gender class and morphometric approach, indicating multivariate allometry. All morphometric approaches yielded significant results, however the hybrid model represented the most complete and powerful model. Our hybrid analyses combined the power of geometric information (spatial covariation between landmarks) with functionally significant measurements of fish areas to fully describe overall morphology. This integrative approach produced a single predator effect canonical axis characterizing both geometric and traditional information simultaneously. We focus on this hybrid model with *G. affinis* and explore its predator effect canonical variate.

The predator effect canonical variate in *G. affinis* explained 63.1% of morphological variation for males, 58.5% for females, and 44.7% for juveniles. Canonical centroids (95% confidence regions) for populations of *G. affinis* along the respective canonical variates for males and females are presented in figure 2. Centroid plots for juveniles are similar and are not shown. Examination of canonical loadings from the hybrid models reveals a consistent shift in morphology between predator regimes for males, females and juveniles (Table 2). The overall effect for predator populations can be summarized from these loadings as five major morphological shifts: 1) deeper body/caudal region and shallower body/head (PW 5Y), 2) longer caudal peduncle (PW 7X), 3) a more elongate body shape for males and juveniles (Uni Y), 4) reduced gonopodium in males (log anal fin area), and 5) lower orientation of the eye and snout (PW 1Y, PW 6Y). The absence of elongation in female *G. affinis* might reflect variation due to gravid state.

In addition to warped grid representation of canonical axes, we further produced for *G. affinis* males a photographic visualization (fig. 3) using tpsSuper (Rohlf 2000*b*). This procedure warped one of our original images using canonical scores from the predator regime canonical axis (hybrid model). Thus the pictures depict morphological differences between predator regimes while controlling for centroid size and population nested within predator regime.

Morphologies differed significantly between ponds and ditches for all age/gender classes (males: $F_{20,119} = 4.63$, $P < 0.0001$; females: $F_{20,113} = 7.03$, $P < 0.0001$; juveniles: $F_{20,115} = 3.31$, $P < 0.0001$). MANCOVAs using residuals from the previous model found significant effects due to predator regime (males: $F_{20,114} = 6.12$, $P < 0.0001$; females: $F_{20,108} = 6.26$, $P < 0.0001$; juveniles: $F_{20,110} = 3.94$, $P < 0.0001$) and population nested within predator regime (males: Wilks' $\Lambda = 0.123$, $F_{80,452.1} = 3.96$, $P < 0.0001$; females: Wilks' $\Lambda = 0.047$, $F_{80,428.5} = 6.27$, $P < 0.0001$, juveniles: Wilks' $\Lambda = 0.139$, $F_{80,436.4} = 3.55$, $P < 0.0001$). Furthermore, the nature of morphological divergence between predator environments (i.e. canonical loadings and thin-plate-spline visualizations) described in the standard hybrid model and the residual hybrid model were extremely similar. So, even after controlling for effects due to pond/ditch, the significance and nature of morphological differences between predator regimes remained unaltered.

Gonopodium size in male *G. affinis* differed between predator regimes ($R^2 = 0.27$, $F_{1,133} = 20.45$, $P < 0.0001$) but not among populations nested within predator regime ($F_{4,133} = 1.91$, $P = 0.11$). Gonopodia (adjusted for centroid size) were 14.6% larger in predator-free populations.

Morphological differences between predator regimes of *G. hubbsi* in the Bahamas were significant for females ($F_{16,27} = 4.49$, $P = 0.0003$), but only marginally significant for males ($F_{16,5} = 4.25$, $P = 0.059$). There was a low sample size of males ($n = 23$), consisting of only six males from the predator-free population. This factor contributed to lower power (5 error degrees of freedom) and likely the failure to observe significance. Multivariate allometry was not evident for females ($F_{16,27} = 1.75$, $P = 0.096$) or males ($F_{16,5} = 3.33$, $P = 0.095$) of *G. hubbsi*. Evaluation of canonical loadings and thin-plate-spline transformations for both males and females of *G. hubbsi* revealed strikingly similar divergence across predator regimes compared with *G. affinis*. So we combined *G. affinis* and *G. hubbsi* in our analyses and found significant differences between predator regimes for both males ($F_{16,139} = 4.62$, $P < 0.0001$) and females ($F_{16,155} = 5.00$, $P < 0.0001$). Population nested within predator regime was also significant for males (Wilks' $\Lambda = 0.028$, $F_{96,794.3} = 7.30$, $P < 0.0001$) and females (Wilks' $\Lambda = 0.021$, $F_{96,885.0} = 8.97$, $P < 0.0001$).

The covariate was significant for both sexes in the combined species models (in both cases $P < 0.0001$).

The predator regime canonical variates from our combined species analyses accounted for 34.7% of morphological variation for males and 34.1% for females. Centroids for populations of *Gambusia* along the respective canonical variates for males and females are presented in figure 4. For males, predator and predator-free populations of *Gambusia* separate along the canonical variate similarly for both species. For males even their placement on this axis is roughly the same for both species. For females, *G. hubbsi* individuals are shifted along the morphological axis, but predator regimes of *G. hubbsi* separate in a similar pattern as *G. affinis*. Specifically, female *G. hubbsi* fall much more into the predator-free region of the axis than do female *G. affinis*, but the nature of divergence between predator regimes is similar for females of both species.

Since predator-free populations of *G. affinis* had larger gonopodia than predator populations, we tested whether this trend held in *G. hubbsi*. Gonopodium size divergence in *G. hubbsi* was similar in magnitude and sign (15.6% larger gonopodium in predator-free population) as for their congener, but was only marginally significant ($R^2 = 0.24$, $F_{1,20} = 3.91$, $P = 0.062$). When gonopodium data of both species were combined in one ANCOVA, divergence in gonopodium size differed significantly across predator regimes ($R^2 = 0.32$, $F_{1,154} = 22.28$, $P < 0.0001$) and population nested within predator regime ($F_{6,154} = 3.32$, $P = 0.004$). Least squares means from this model demonstrate that gonopodia (adjusted for centroid size) of *G. hubbsi* are larger than gonopodia of *G. affinis* but differ in size between predator regimes in a similar way (fig. 5).

Shape of *P. reticulata* differed between predator regimes ($F_{18,45} = 3.98$, $P < 0.0001$) but not population nested within predator regime (Wilks' $\Lambda = 0.431$, $F_{36,90} = 1.31$, $P = 0.156$). Also, there was no evidence of multivariate allometry ($F_{18,45} = 1.03$, $P = 0.456$). Examination of the predator regime canonical axis loadings (61% of shape variance) revealed that the overwhelming morphological shift was a more elongate body shape in predator populations (see also Endler 1980; C.K. Ghalambor, D.N. Reznick and J.A. Walker unpublished data). Our combined analysis, which simultaneously analyzed morphologies of all three species, found significant divergence between predator regimes ($F_{18,200} = 4.52$, $P < 0.0001$) and population nested within predator regimes (Wilks' $\Lambda = 0.014$, $F_{180,1779.5} = 6.23$, $P < 0.0001$). The predator regime canonical axis described 29% of shape variance. That is, an axis explaining 29% of shape variance describes a shift between predator regimes shared by all species. Examination of canonical loadings revealed a morphological shift similar to that found in analyses of *G. affinis* alone. Four of the largest loadings described a deeper and longer body/caudal region, shallower body/head and more elongate body shape in predator populations. Canonical centroids for predator regimes of each species are presented in figure 6. Centroid placement on the axis demonstrates that males of all three species responded similarly to predator regime, however different species resided in different regions of the axis.

Step one of our two-step model to examine species-free aspects of divergence found strong shape differences among species (Wilks' $\Lambda = 0.093$, $F_{36,420} = 26.55$, $P < 0.0001$). Residuals from the first model were entered into the second step. This MANCOVA (using "species free" residuals) found significant morphological differences between predator regimes ($F_{18,200} = 4.52$, $P < 0.0001$) and population nested within predator regime (Wilks' $\Lambda = 0.151$, $F_{180, 1779.5} = 2.38$, $P < 0.0001$). Our main interest here was to facilitate study of the unique and shared aspects of divergence among species. Indeed, the first three canonical variates of this model appeared to partition these aspects of divergence (fig. 7). The first canonical axis (CA 1) explained 52% of shape variance and primarily described divergence between predator regimes for *P. reticulata*. CA 2 explained 43% of the shape variance and characterized divergence for *G. affinis*. CA 3 explained 37% of the shape variance and described aspects of morphological divergence between predator regimes that were shared across all species, but emphasized the shift for *G. hubbsi*.

Our discriminant function analysis indicated differentiation across predator regimes ($F_{18,211} = 3.89$, $P < 0.0001$) and classified 165 of 230 fish (72%) into correct predator regimes. Thus morphological responses to predation are similar enough among species to obtain reasonable discrimination without adjusting for species-level shapes or species-specific responses. With a species effect in this model DFA correctly classified 82% of fish by predator regime (Wilks' $\Lambda = 0.032$, $F_{90,1008.7} = 11.53$, $P < 0.0001$).

Discussion

Diversification across predator regimes was strongly apparent in this study. We documented both a general response and species-specific responses to predation. The general effect involved a shift in allocation from the body/head region to the caudal box region and overall elongation of the body in predatory environments (fig. 6; CA 3 in fig. 7). Species-specific aspects of divergence were largely due to cross-genus differences (*Poecilia* versus *Gambusia*). For example, the response of *Poecilia* unshared by *Gambusia* was elongation of the entire body rather than mostly localized elongation of the peduncle region (CA 1 in fig. 7). The unique feature of divergence for *G. affinis* (versus *Poecilia* and *G. hubbsi*) seemed mainly to involve a pointed snout and lower eye position in the presence of predators (fig. 2, CA 2 in fig. 7). Species-specific differences in response are taken to reflect unmeasured environmental differences experienced by each species, such as hydrological differences or distinctive aspects of local predators, or random differences that do not reflect adaptation. We focus on the shared elements of divergence for they likely reflect a generalized adaptive solution to the measured predator gradients.

The generalized pattern of morphological divergence persisted across multiple species, age/gender classes, specific predator regimes and geographic regions. For example, in *G. affinis* shape differentiation between predator regimes was similar for each age/gender class (Table 2). Without even entering species identity into our statistical models (i.e. all populations treated equally), 29% of shape variance was attributable to a generalized response to predators. This trend held despite radical differences in predator regimes and physical environments across species. For example, predators in our various poeciliid populations ranged from centrarchid sunfish to cichlids to needlefish and barracuda. Such generality strongly suggests that DNS (i.e. fitness trade-offs) created by piscivorous fish is largely invariant across phylogenetic and environmental boundaries. We note that generalized morphological differences between predator regimes do not demonstrate divergent natural selection, but do present evidence that DNS may be operating.

The nature of shared morphological change across predator regimes may reflect the functional ecology of swimming. Fast starts and powered turns, maneuvers that can be used for predator avoidance, are generated by the posterior body/caudal fin portion of fishes (Webb 1984). These forms of locomotion increase survival in many prey fishes (Howland 1974; Webb

1982; Weihs and Webb 1983; Webb 1986). Preliminary performance trials with a subset of *G. affinis* males from the present study suggest greater escape ability among individuals with predator-associated morphology. Fish with high predator-regime canonical scores had greater escape scores, where escape was defined as the product of velocity and distance traveled ($R^2 = 0.39$, $P = 0.023$, $n = 13$; M. C. Belk, D. Gonzales, R. B. Langerhans, T. J. DeWitt, unpublished data). In contrast, predator-associated morphology reduced endurance swimming ($R^2 = 0.38$, $P = 0.024$, $n = 13$; ibidem). Endurance reductions reflect hydrodynamic costs (Webb 1993). Such costs should only be assumed when compensatory benefits exist. Here, predator-associated morphology appears to offer benefits only in the presence of predators (i.e. increased escape ability) but energetic costs in all environments. If predator-associated morphology were cost-free (e.g. no endurance reduction), then we would expect fish in all populations to exhibit this morphology (Via and Lande 1985; Moran 1992). This tradeoff seems like a likely candidate to produce DNS. A similar situation seems to apply for crucian carp. In these fish predator-associated morphology increases survivorship in the presence of predators but entails a hydrodynamic cost (Brönmark and Miner 1992; Nilsson et al. 1995; Pettersson and Brönmark 1997, 1999).

An interesting aspect of divergence between predator regimes involved gonopodium size, however the nature of response differed across genera. Gonopodia were relatively smaller in the presence of predators for both *G. affinis* and *G. hubbsi* (fig. 5). The nature of this divergence suggests that a larger intromittent organ may be beneficial for reproductive success (e.g. increased insemination efficiency, mate choice), but prove costly in predator environments (e.g. increased drag, visibility to predators, restricted maneuverability). The pattern of gonopodia size differences in *Gambusia* is opposite that found in *P. reticulata*. While we could not measure gonopodium size for *P. reticulata* in this study, Kelly et al. (2000) documented longer gonopodia in predator populations of *P. reticulata*. Kelly et al. (2000) proposed that longer gonopodia facilitate gonopodial thrusting (sneaky mating), and thus represent an adaptive change resulting from a shift in mating tactics in the presence of predators (sneaky mating increases with predator intensity in guppies, Farr 1975). However, no studies support a functional basis for longer gonopodia increasing gonopodial thrusting success. In fact, smaller males (thus small gonopodia) are more successful in inseminating females using gonopodial thrusting in many poeciliids, including guppies and *Gambusia* (Bisazza and Marin 1995; Bisazza and Pilastro 1997; Pilastro et al. 1997). Although we are uncertain as to the specific mechanisms responsible for the divergence in gonopodium size across the predation gradient, it represents an interesting example of both convergence and divergence within a clade. The nature of trends in gonopodia size and their relationship to mating and life history strategies warrant further study.

Comparing responses of multiple species to an environmental gradient is a key way to define general mechanisms of diversification (Cody and Mooney 1978; Winemiller 1991). For example, Endler documented largely convergent patterns of body color differences across predator regimes for guppies (Endler 1978) and *Phalloceros caudomaculatus* (Endler 1982). Both species exhibited more color in the absence of predators. Similarly, Johnson & Belk (2001) demonstrated predator-associated life-history divergence in *Brachyraphis rhabdophora* that converged on a pattern previously found in guppies (Reznick & Endler 1982). Both species exhibited maturity at smaller size and produced more and smaller offspring in the presence of predators. These cases are similar to the present study in that shared patterns of divergence across a gradient are measured for multiple species. In the present study however, we focus on multiple species in a single study and array the species in the same trait space.

Although similarities across taxa in their response to a common environmental axis can elucidate general mechanisms of DNS, these similar patterns of divergence are only half the story. The way species differ in their response is also interesting. In theory we predict that where multiple species experience similar selection regimes, their evolutionary responses will involve both shared and unique features. Several studies have emphasized the shared aspects of divergence ("convergent divergence") but few have rendered and visualized both shared and unique elements. Our results, taken with Endler (1982) and Johnson & Belk (2001), suggest that predation has general effects on multiple traits across phylogenetically distinct species (Parenti and Rauchenberger 1989; Lydeard et al. 1995*a*, *b*).

Either phenotypic plasticity or constitutive adaptation may underlie population differences, but both could represent evolutionary responses to DNS. It is widely accepted that constitutive adaptations to local environmental conditions produce diversification and speciation (Rice and Hostert 1993; Skulason and Smith 1995; Orr and Smith 1998; Schluter 2000*b*). However, plasticity may also promote diversification and facilitate evolution of constitutive adaptations (Stearns 1989; West-Eberhard 1989; Meyer 1990; Wimberger 1991; Losos et al. 2000; Agrawal 2001). In the present study we don't know the relative contributions of constitutive differences versus plasticity in the observed levels of diversification. In some other fish species, diversification has stemmed from both mechanisms simultaneously (Day et al. 1994; Robinson and Wilson 1996; Chapman et al. 2000).

Whether the shared nature of response resulted from multiple evolutionary events, or from a single adaptation in an ancestral species is not known. For example, shared patterns of divergence across predator regimes by multiple species could represent a single ancestral adaptation of phenotypic plasticity that all species retain in more or less modified form.

However, species-specific (unshared) responses likely represent more recent divergence and support the concept that there are multiple ways to escape predation.

Although we focused on responses of poeciliid fish to alternative predator environments, the concept of defining shared and unique axes of variation across a selection gradient applies broadly. For example, many plants exhibit a shade-avoidance reaction (i.e. stem elongation in response to foliar shade). Though this basic response is shared by many species, some populations within these species exhibit convergent patterns of uniquely reduced responses due to local adaptation. For velvetleaf (*Ambutilon theophrasti*) in corn fields, stem elongation does not allow the plants to overtop corn, so reduced responses are adaptive to reduce stem breakage (Weinig 2000). Likewise, woodland *Impatiens capensis* populations have a reduced response reflecting their inability to overtop trees (Dudley and Schmitt 1996). So while many features of response to foliar shading are shared between species and populations, unique exceptions arise due to localized environmental differences.

There has been increasing awareness of the need to evaluate ecological patterns in an evolutionary context (McPeek and Miller 1996). The present study demonstrates patterns of population divergence within and between species that are likely produced and maintained via ecological trade-offs (DNS). Such trade-offs have profound implications for evolutionary divergence and maintenance of body morphologies and other species characteristics (e.g. behavior, life history). While it is important to define similarities in response to DNS across species to elucidate instances of convergence, explicit characterization of species-specific responses may also be important. In particular, species-specific deviations from a general trend may indicate cases of localized peculiarities worthy of investigation.

Acknowledgments

We thank Heather Prestridge, Albrey Arrington and Brian Silliman for their assistance collecting specimens and sampling fish communities. We also thank Mark Belk and David Rodriguez for conducting preliminary performance trials with fish from this study. Helpful comments on early versions of this manuscript were provided by Kirk Winemiller, Albrey Arrington and Brian Silliman.

Literature Cited

Agrawal, A. A. 2001. Phenotypic plasticity in the interactions and evolution of species. Science 294:321-326.

Belk, M. C. 1998. Predator-induced delayed maturity in bluegill sunfish (*Lepomis macrochirus*): variation among populations. Oecologia 113:203-209.

Bennington, C. C., and W. V. Thayne. 1994. Use and misuse of mixed-model analysis of variance in ecological studies. Ecology 75:717-722.

Bisazza, A., and G. Marin. 1995. Sexual selection and sexual size dimorphism in the Eastern mosquitofish *Gambusia holbrooki* (Pisces: Poeciliidae). Ethology Ecology & Evolution 7:169-183.

Bisazza, A., and A. Pilastro. 1997. Small male mating advantage and reversed size dimorphism in poeciliid fishes. Journal of Fish Biology 50:397-406.

Bookstein, F. L. 1991. Morphometric tools for landmark data. Cambridge University Press, New York.

Brönmark, C., and J. G. Miner. 1992. Predator-induced phenotypical change in body morphology in crucian carp. Science 258:1348-1350.

Chapman, L. J., F. Galis, and J. Shinn. 2000. Phenotypic plasticity and the possible role of genetic assimilation: hypoxia-induced trade-offs in the morphological traits of an African cichlid. Ecology Letters 3:387-393.

Cody, M. L., and H. A. Mooney. 1978. Convergence versus nonconvergence in Mediterranean-climate ecosystems. Annual Review of Ecology and Systematics 9:265-321.

Day, T., J. Pritchard, and D. Schluter. 1994. A comparison of two sticklebacks. Evolution 48:1723-1734.

DeWitt, T. J., B. W. Robinson, and D. S. Wilson. 2000. Functional diversity among predators of a freshwater snail imposes an adaptive trade-off for shell morphology. Evolutionary Ecology Research 2:129-148.

Douglas, M. E., M. R. Douglas, J. M. Lynch, and D. M. McElroy. 2001. Use of geometric morphometrics to differentiate *Gila* (Cyprinidae) within the upper Colorado River basin. Copeia:389-400.

Downhower, J. F., L. P. Brown, and M. L. Matsui. 2000. Life history variation in female *Gambusia hubbsi*. Environmental Biology of Fishes 59:415-428.

Dudley, S. A., and J. Schmitt. 1996. Genetic differentiation and morphological responses to simulated foliage shade between populations of *Impatiens capensis* from open and woodland sites. Functional Ecology 9:655-666.

Endler, J. A. 1978. A predator's view of animal color patterns. Evolutionary Biology 11:319-364.

———. 1980. Natural selection on color patterns in *Poecilia reticulata*. Evolution 34:76-91.

———. 1982. Convergent and divergent effects of natural selection on color patterns in two fish faunas. Evolution 36:178-188.

———. 1983. Natural and sexual selection on color patterns in poeciliid fishes. Environmental Biology of Fishes 9:173-190.

———. 1995. Multiple-trait coevolution and environmental gradients in guppies. Trends in Ecology and Evolution 10:22-29.

Farr, J. A. 1975. Role of predation in evolution of social behavior of natural populations of the guppy, *Poecilia reticulata* (Pisces: Poeciliidae). Evolution 29:151-158.

Harvell, C. D. 1986. The ecology and evolution of inducible defenses in a marine bryozoan: cues, costs, and consequences. American Naturalist 128:810-823.

Havel, J. E. 1987. Predator-induced defenses: a review. Pages 263-278 *in* W. C. Kerfoot, and A. Sih, eds. Predation: direct and indirect impacts on aquatic communities. University Press of New England, Hanover, N.H.

Houde, A. E. 1997. Sex, color, and mate choice in guppies. Princeton University Press, Princeton, N.J.

Howland, H. C. 1974. Optimal strategies for predator avoidance: The relative importance of speed and maneuverability. Journal of Theoretical Biology 47:333-350.

Jackson, D. A., P. R. Peres-Neto, and J. D. Olden. 2001. What controls who is where in freshwater fish communities - the roles of biotic, abiotic, and spatial factors. Canadian Journal of Fisheries and Aquatic Sciences 58:157-170.

Johnson, J. B., and M. C. Belk. 2001. Predation environment predicts divergent life-history phenotypes among populations of the livebearing fish *Brachyrhaphis rhabdophora*. Oecologia 126:142-149.

Kelly, C. D., J. G. J. Godin, and G. Abdallah. 2000. Geographical variation in the male intromittent organ of the Trinidadian guppy (*Poecilia reticulata*). Canadian Journal of Zoology 78:1674-1680.

Kerfoot, W. C., and A. Sih. 1987. Predation : direct and indirect impacts on aquatic communities. University Press of New England, Hanover, N.H.

Krumholz, L. 1963. Relationships between fertility, sex ratio, and exposure to predation in populations of the mosquitofish, *Gambusia manni* at Bimini, Bahamas. Internationale Revue der gesamten Hydrobiologie 48:201-256.

Lardner, B. 2000. Morphological and life history responses to predators in larvae of seven anurans. Oikos 88:169-180.

Layman, C. A., and B. R. Silliman. in press. Preliminary survey and diet analysis of juvenile fishes of an estuarine creek on Andros Island, Bahamas. Bulletin of Marine Science.

Levins, R. 1968. Evolution in changing environments. Princeton University Press, New Jersey.

Losos, J. B., D. A. Creer, D. Glossip, R. Goellner, A. Hampton, G. Roberts, N. Haskell, P. Taylor, and J. Ettling. 2000. Evolutionary implications of phenotypic plasticity in the hindlimb of the lizard *Anolis sagrei*. Evolution 54:301-305.

Lydeard, C., M. C. Wooten, and A. Meyer. 1995*a*. Cytochrome-B sequence variation and a molecular phylogeny of the live-bearing fish genus *Gambusia* (Cyprinodontiformes, Poeciliidae). Canadian Journal of Zoology 73:213-227.

———. 1995*b*. Molecules, morphology, and area cladograms - a cladistic and biogeographic analysis of *Gambusia* (Teleostei, Poeciliidae). Systematic Biology 44:221-236.

Magurran, A. E., and R. M. May. 1999. Evolution of biological diversity. Oxford University Press, Oxford.

McPeek, M. A., and T. E. Miller. 1996. Evolutionary biology and community ecology. Ecology 77:1319-1320.

Meecham, C. A. 1993. MorphoSys: an interactive machine vision program for acquisition of morphometric data. Pages 393-402 *in* R. Fortuner, ed. Advances in computer methods for systematic biology. Artificial intelligence, databases, computer vision. Johns Hopkins University Press, Baltimore, M.D.

Meffe, G. K., and F. F. Snelson. 1989*a*. An ecological overview of poeciliid fishes *in* G. K. Meffe, and F. F. Snelson, eds. Ecology and Evolution of Livebearing Fishes (Poeciliidae). Prentice Hall, Englewood Cliffs, N.J.

———. 1989*b*. Ecology and evolution of livebearing fishes (Poeciliidae). Prentice Hall, Englewood Cliffs, N.J.

Meffe, G. K., S. C. Weeks, M. Mulvey, and K. L. Kandl. 1995. Genetic differences in thermal tolerance of eastern mosquitofish (*Gambusia holbrooki* Poeciliidae) from ambient and thermal ponds. Canadian Journal of Fisheries and Aquatic Sciences 52:2704-2711.

Meyer, A. 1990. Ecological and evolutionary consequences of the trophic polymorphism in *Cichlasoma citrinellum* (Pisces, Cichlidae). Biological Journal of the Linnean Society 39:279-299.

Moran, N. A. 1992. The evolutionary maintenance of alternative phenotypes. American Naturalist 139:971-989.

Nagelkerken, I., S. Kleijnen, T. Klop, R. van den Brand, E. C. de la Moriniere, and G. van der Velde. 2001. Dependence of Caribbean reef fishes on mangroves and seagrass beds as nursery habitats: a comparison of fish faunas between bays with and without mangroves/seagrass beds. Marine Ecology Progress Series 214:225-235.

Nilsson, P. A., C. Brönmark, and L. B. Pettersson. 1995. Benefits of a predator-induced morphology in crucian carp. Oecologia 104:291-296.

Orr, M. R., and T. B. Smith. 1998. Ecology and speciation. Trends in Ecology & Evolution 13:502-506.

Parenti, L. R., and M. Rauchenberger. 1989. Systematic overview of the poeciliines. Pages 3-12 *in* G. K. Meffe, and F. F. Snelson, eds. Ecology and evolution of livebearing fishes (Poeciliidae). Prentice Hall, Englewood Cliffs, N.J.

Pettersson, L. B., and C. Brönmark. 1997. Density-dependent costs of an inducible morphological defense in Crucian carp. Ecology 78:1805-1815.

———. 1999. Energetic consequences of an inducible morphological defence in crucian carp. Oecologia 121:12-18.

Pilastro, A., E. Giacomello, and A. Bisazza. 1997. Sexual selection for small size in male mosquitofish (*Gambusia holbrooki*). Proceedings of the Royal Society of London B, Biological Sciences 264:1125-1129.

Relyea, R. A. 2001. Morphological and behavioral plasticity of larval anurans in response to different predators. Ecology 82:523–540.

Reznick, D. N. 1989. Life-history evolution in guppies 2. Repeatability of field observations and the effects of season on life histories. Evolution 43:1285-1297.

———. 1996. Life history evolution in guppies: A model system for the empirical study of adaptation. Netherlands Journal of Zoology 46:172-190.

Reznick, D. N., and H. A. Bryga. 1996. Life-history evolution in guppies (*Poecilia reticulata*: Poeciliidae). V. Genetic basis of parallelism in life histories. American Naturalist 147:339-359.

Reznick, D. N., M. J. Butler, and H. Rodd. 2001. Life-history evolution in guppies. VII. The comparative ecology of high- and low-predation environments. American Naturalist 157:126-140.

Reznick, D. N., and J. A. Endler. 1982. The impact of predation on life history evolution in Trinidadian guppies (*Poecilia reticulata*). Evolution 36:160-177.

Reznick, D. N., F. H. Shaw, F. H. Rodd, and R. G. Shaw. 1997. Evaluation of the rate of evolution in natural populations of guppies (*Poecilia reticulata*). Science 275:1934-1937.

Rice, W. R., and E. E. Hostert. 1993. Laboratory experiments on speciation: What have we learned in 40 years? Evolution 47:1637-1653.

Robinson, B. W., and D. S. Wilson. 1994. Character release and displacement in fishes: A neglected literature. American Naturalist 144:596-627.

———. 1995. Experimentally induced morphological diversity in Trinidadian guppies (*Poecilia reticulata*). Copeia 2:294-305.

———. 1996. Genetic variation and phenotypic plasticity in a trophically polymorphic population of pumpkinseed sunfish (*Lepomis gibbosus*). Evolutionary Ecology 10:631-652.

Robinson, B. W., D. S. Wilson, and G. O. Shea. 1996. Trade-offs of ecological specialization: An intraspecific comparison of pumpkinseed sunfish. Ecology 77:170-178.

Rodd, F. H., and D. N. Reznick. 1997. Variation in the demography of guppy populations: The importance of predation and life histories. Ecology 78:405-418.

Rodd, F. H., D. N. Reznick, and M. B. Sokolowski. 1997. Phenotypic plasticity in the life history of guppies: responses to social environment. Ecology 78:419-433.

Rohlf, F. J. 2000*a*. TpsRegr. Version 1.24. Department of Ecology and Evolution, State Univ. New York, Stony Brook, N.Y.

———. 2000*b*. TpsSuper. Version 1.07. Department of Ecology and Evolution, State Univ. New York, Stony Brook, N.Y.

———. 2001. TpsRelw, Version 1.24. Department of Ecology and Evolution, State Univ. New York, Stony Brook, N.Y.

Rohlf, F. J., and L. F. Marcus. 1993. A revolution in morphometrics. Trends in Ecology & Evolution 8:129-132.

Rosen, D. E., and R. M. Bailey. 1963. The poeciliid fishes (Cyprinodontiformes), their structure, zoogeography, and systematics. Bulletin of the American Museum of Natural History 126:1-176.

Schluter, D. 2000*a*. Ecological character displacement in adaptive radiation. American Naturalist 156:S4-S16.

———. 2000*b*. The Ecology of Adaptive Radiation. Oxford University Press, Oxford.

Seghers, B. H. 1973. Analysis of geographic variation in the antipredator adaptations of the guppy, *Poecilia reticulata*. Ph.D. thesis, University of British Columbia.

———. 1974. Schooling behavior in the guppy (*Poecilia reticulata*): An evolutionary response to predation. Evolution 28:486-489.

Sih, A., P. H. Crowley, M. A. McPeek, J. Petranka, and K. Strohmeier. 1985. Predation, competition, and prey communities. Annual Review of Ecology and Systematics 16:269-311.

Skelly, D. K., and E. E. Werner. 1990. Behavioral and life historical responses of larval American toads to an odonate predator. Ecology 71:2313-2322.

Skulason, S., and T. B. Smith. 1995. Resource polymorphisms in vertebrates. Trends in Ecology & Evolution 10:366-370.

Smith, T. B., and S. Skulason. 1996. Evolutionary significance of resource polymorphisms in fishes, amphibians, and birds. Annual Review of Ecology and Systematics 27:111-133.

Sohn, J. J. 1977. Consequences of predation and competition upon demography of *Gambusia manni* (Pisces: Poeciliidae). Copeia 1977:224-227.

Sokal, R. R., and F. J. Rohlf. 1995. Biometry. Freeman, New York.

Stearns, S. C. 1989. The evolutionary significance of phenotypic plasticity. Bioscience 39:436-445.

Tollrian, R., and C. D. Harvell. 1999. The ecology and evolution of inducible defenses. Princeton University Press, Princeton, N.J.

Trussell, G. C. 2000. Predator-induced plasticity and morphological trade-offs in latitudinally separated populations of *Littorina obtusata*. Evolutionary Ecology Research 2:803-822.

Van Buskirk, J., S. A. McCollum, and E. E. Werner. 1997. Natural selection for environmentally induced phenotypes in tadpoles. Evolution 54:1983-1992.

Van Buskirk, J., and B. R. Schmidt. 2000. Predator-induced phenotypic plasticity in larval newts: trade-offs, selection, and variation in nature. Ecology 81:3009-3028.

Van Valkenburgh, B., and R. K. Wayne. 1994. Shape divergence associated with size convergence in sympatric East African jackals. Ecology 75:1567-1581.

Via, S., and R. Lande. 1985. Genotype-environment interaction and the evolution of phenotypic plasticity. Evolution 39:505-522.

Walker, J. A. 1997. Ecological morphology of lacustrine threespine stickleback *Gasterosteus aculeatus* L. (Gasterosteidae) body shape. Biological Journal of the Linnean Society 61:3-50.

Walker, J. A., and M. A. Bell. 2000. Net evolutionary trajectories of body shape evolution within a microgeographic radiation of threespine sticklebacks (*Gasterosteus aculeatus*). Journal of Zoology 252:293-302.

Webb, P. W. 1982. Avoidance responses of fathead minnow to strikes by four teleost predators. Journal of Comparative Physiology. 147A:371-378.

———. 1984. Body form, locomotion, and foraging in aquatic vertebrates. American Zoologist 24:107-120.

———. 1986. Effect of body form and response threshold on the vulnerability of four species of teleost prey attacked by largemouth bass (*Micropterus salmoides*). Canadian Journal of Fisheries and Aquatic Sciences 43:763-771.

———. 1993. Swimming. Pages 47-73 *in* D. H. Evans, ed. The physiology of fishes. CRC, Boca Raton, Fla.

Weihs, D., and P. W. Webb. 1983. Optimization of locomotion *in* P. W. Webb, and D. Weihs, eds. Fish biomechanics. Praeger, New York.

Weinig, C. 2000. Differing selection in alternative competitive environments: shade-avoidance responses and germination timing. Evolution 54:124-136.

West-Eberhard, M. J. 1989. Phenotypic plasticity and the origins of diversity. Annual Review of Ecology and Systematics 20:249-278.

Wimberger, P. H. 1991. Plasticity of jaw and skull morphology in the neotropical cichlids *Geophagus brasiliensis* and *G. steindachneri*. Evolution 45:1545-1563.

Winemiller, K. O. 1991. Ecomorphological diversification in lowland freshwater fish assemblages from five biotic regions. Ecological Monographs 61:343-365.

Winemiller, K. O., M. Leslie, and R. Roche. 1990. Phenotypic variation in male guppies from natural inland populations: an additional test of Haskins' sexual selection/predation hypothesis. Environmental Biology of Fishes 29:179-191.

Table 1: Results of MANCOVAs examining shape variation among populations of *Gambusia affinis*.

Morphometric approach	Effects	Males (N = 140)			Females (N = 134)			Juveniles (N = 136)		
		F	df	*P*	*F*	df	*P*	*F*	df	*P*
Traditional	Predator regime	6.59	12,122	<0.0001	8.80	12,116	<0.0001	6.11	12,118	<0.0001
	Population(Predator regime)*	4.53	48,472.0	<0.0001	5.20	48,448.9	<0.0001	3.62	48,456.6	<0.0001
Geometric	Predator regime	10.73	16,118	<0.0001	6.72	16,112	<0.0001	5.32	16,114	<0.0001
	Population(Predator regime)*	4.49	64,464.2	<0.0001	5.10	64,440.7	<0.0001	4.24	64,448.6	<0.0001
Hybrid	Predator regime	9.73	20,114	<0.0001	7.61	20,108	<0.0001	4.45	20,110	<0.0001
	Population(Predator regime)*	4.06	80,452.1	<0.0001	4.68	80,428.5	<0.0001	3.53	80,436.4	<0.0001

* *F* is approximated using Wilks' Lambda values.

Note: Shape data for the traditional approach were 12 traditional morphometric measurements (see text; fig. 1a). For the geometric approach 16 partial warps (see text) were used. The hybrid approach used the 16 partial warps plus four area variables (see text).

Table 2: Canonical loadings of the predator effect from hybrid models with *Gambusia affinis*.

Dependent Variable	Interpretation	Males	Females	Juveniles
PW 1X	eye - snout length	0.1741	0.0395	0.0131
PW 1Y	**eye position**	**-0.1031**	**-0.3492**	**-0.2849**
PW 2X	base of the anal fin	**0.2493**	0.0429	-0.0423
PW 2Y	posterior anal fin insertion	**-0.2196**	0.0023	-0.1054
PW 3X	base of dorsal and anal fins	0.0919	0.0835	0.0251
PW 3Y	posterior dorsal and anal insertions	-0.1967	-0.0230	-0.1700
PW 4X	not interpretable	0.0879	0.0332	0.1157
PW 4Y	caudal peduncle depth	-0.0550	-0.0901	**0.3080**
PW 5X	not interpretable	-0.0426	-0.0746	-0.0890
PW 5Y	**shallowness of head / deepness of caudal box**	**0.5475**	**0.3874**	**0.1921**
PW 6X	head length	**-0.2344**	0.0260	-0.1368
PW 6Y	**snout position**	**-0.1443**	**-0.1210**	**-0.3729**
PW 7X	**caudal peduncle length**	**0.2155**	**0.3743**	**0.2147**
PW 7Y	back-archedness	0.0142	0.0291	0.1405
Uniform X	shearing	-0.0782	-0.1203	-0.0856
Uniform Y	**aspect ratio (elongateness)**	**0.1753**	**-0.0598**	**0.5488**
Dorsal fin	logarithm of dorsal fin area	-0.0191	0.1821	0.0759
Caudal fin	logarithm of caudal fin area	-0.0199	**0.2813**	-0.0012
Anal fin	logarithm of anal fin area	**-0.2950**	0.1480	0.0126
Caudal box	logarithm of caudal box area	0.1911	0.1071	-0.0452

Note: Interpretations describe the nature of respective partial warps and areas. Partial warps were interpreted by examining animations in tpsRelw (Rohlf 2001). All loadings greater than 0.2 are in bold type. If the average loading for all age/gender classes is greater than 0.2 then the entire row is bold.

Figure 1: Landmarks used for morphometric analyses. (a) *Gambusia affinis* male showing landmarks used in traditional, geometric, and hybrid morphometric analyses for this species. The traditional morphometric approach used lateral lengths: snout (1-10), caudal peduncle (average of 4-5, 6-7), caudal fin (11-12) and standard length (1-11); body depths: head (2-9), midbody (3-7) and caudal peduncle depth (5-6); ratio data: aspect ratio (1-11 / line halfway along and perpendicular to line 1-11); and areas: total (entire body area including fins), caudal box (box from enclosed connection of 4-5-6-7, shaded region above), dorsal fin (isolated fin above 3-4), caudal fin (isolated fin right of 5-6) and anal fin area (isolated fin below 7-8). Landmarks 1-10 were used for geometric morphometric and hybrid approaches. (b) Landmarks for the medial axis technique applied to all species (male *G. hubbsi* depicted). This technique allowed comparison of *P. reticulata* with *Gambusia* species, as landmarks in (a) could not be digitized in guppies.

Figure 2: Populations of *Gambusia affinis* males and females along their respective predator-effect canonical axes. Ellipses represent 95% confidence centroids of each population (filled symbols indicate predator populations and open symbols indicate predator-free populations; P_1 = Krenek Tap, P_2 = Riverside Campus A, P_3 = University Oaks, NP_1 = Hensel, NP_2 = Riverside Campus B, NP_3 = Autumn Circle). Males and females were analyzed separately, however values were scaled to canonical means to depict both genders in one panel. Visualizations of the canonical shape axis is provided above the figure for males and below for females. Thin-plate-spline transformation grids (magnified 3x) were generated using landmark coordinates and canonical scores in tpsRegr (Rohlf 2000). Juvenile centroids are qualitatively similar and are not presented.

Figure 3: Photographic representation of morphological divergence between predator regimes for *G. affinis* males. The images represent landmark configurations on either end of the predator regime canonical axis (hybrid model, magnified 3x) applied to a single photograph of an individual with an intermediate canonical score.

Figure 4: Populations of *G. affinis* and *G. hubbsi* across gender-specific predation axes. Filled symbols indicate predator populations and open symbols indicate predator-free populations. Population numbers follow figure 2. Ellipses represent 95% confidence centroids for each population. Species were analyzed together but are presented on separate lines for clarity. Thin-plate-spline transformation grids illustrate the shape change over each axis (1x magnification). (a) Shape gradient in males. (b) Shape gradient in females.

Figure 5: Gonopodium size divergence among populations of *Gambusia affinis* and *G. hubbsi*. Population numbering follows figure 2. Plotted are least squares means for each population ± 1 SE.

Figure 6: Centroids (95% confidence regions) for each species along the predator effect canonical axis. For clarity, population centroids were averaged by species within predator regime (thus, only two centroids are given for each species). Filled symbols indicate predator populations (P) and open symbols indicate predator-free populations (NP). Transformation grids are given to visualize shape change over this gradient (1x magnification).

Figure 7: The first three canonical axes of a “species-free” MANCOVA. Filled symbols indicate predator populations (P) and open symbols indicate predator-free populations (NP). Transformation grids illustrate changes on each axis (magnified 2x). Convex hulls (shaded triangles) were projected onto the CA 1/CA 3 plane, to help visualize the shared nature of divergence across predator regimes on CA 3.

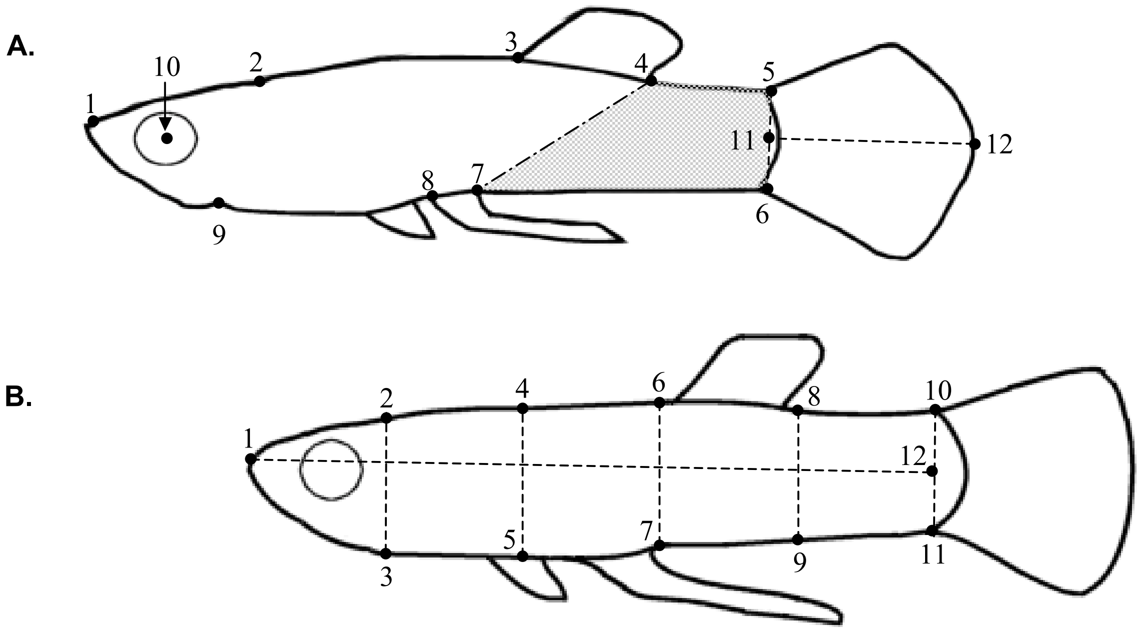

Figure 1

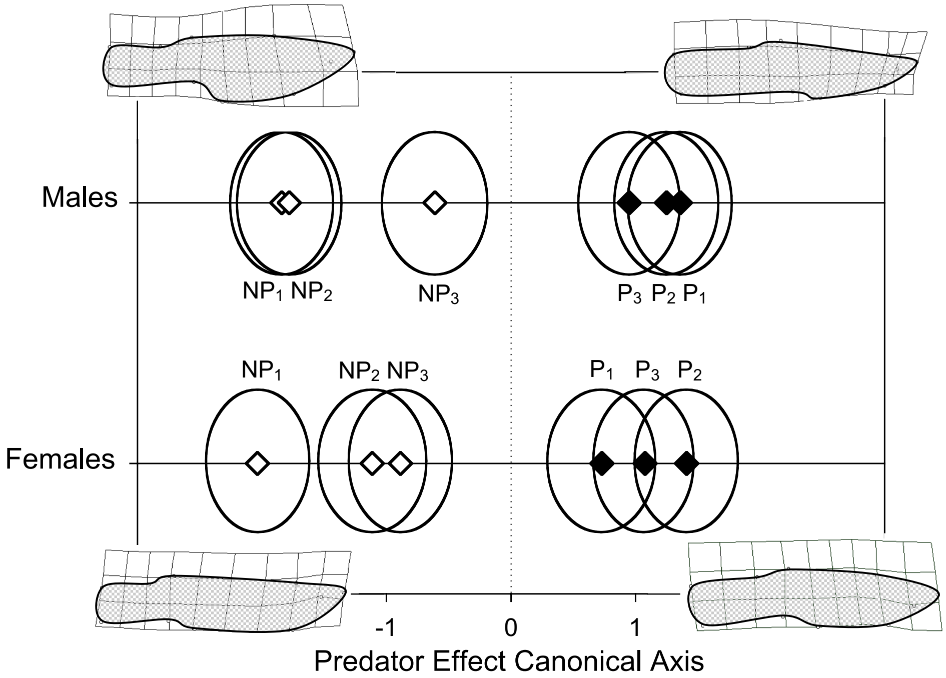
Males
NP1 NP2
NP3
P3 P2 P1
NP1
NP2 NP3
P1 P3 P2
Females
-1
0
1
Predator Effect Canonical Axis

Figure 2

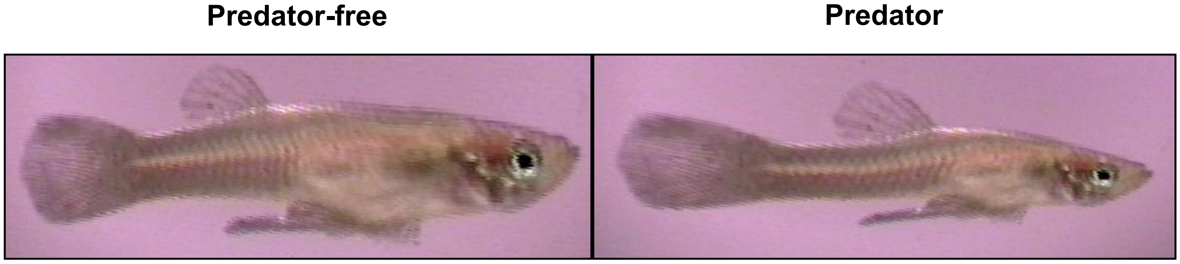

Figure 3

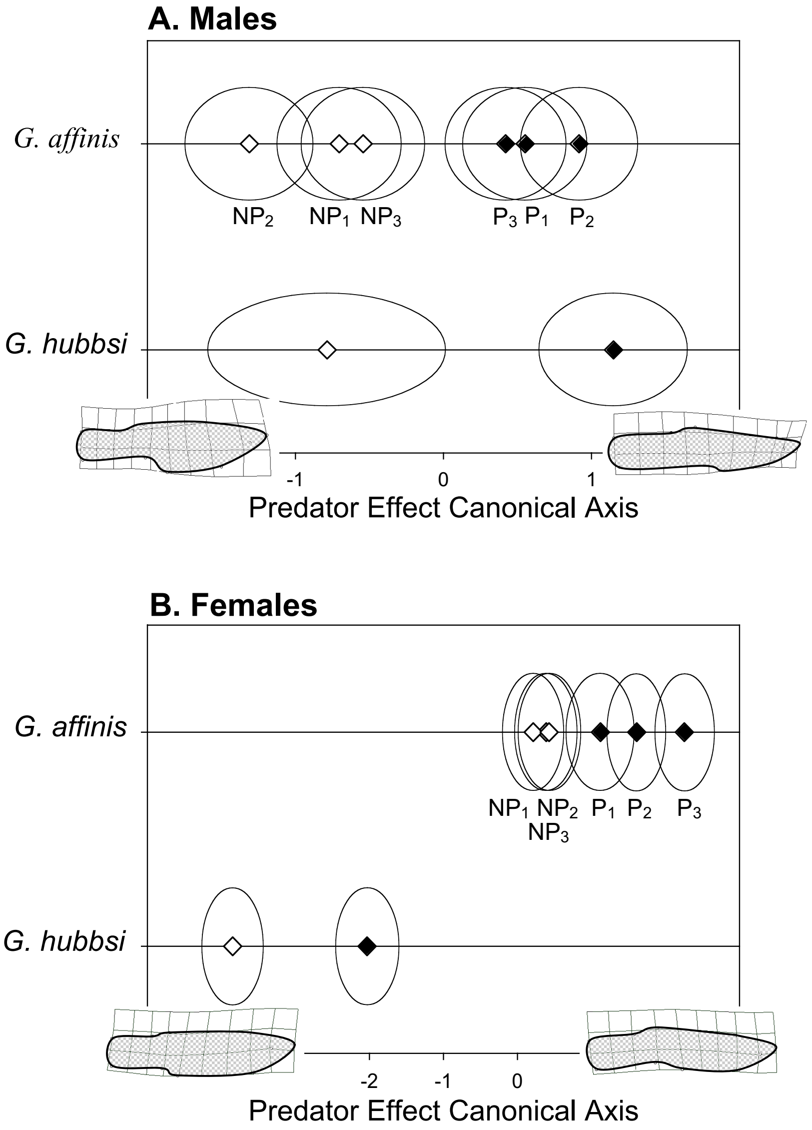
A. Males
G. affinis
NP2
NP1
NP3
P3
P1
P2
G. hubbsi
-1
0
1
Predator Effect Canonical Axis
B. Females
G. affinis
NP1
NP2
NP3
P1
P2
P3
G. hubbsi
-2
-1
0
Predator Effect Canonical Axis

Figure 4

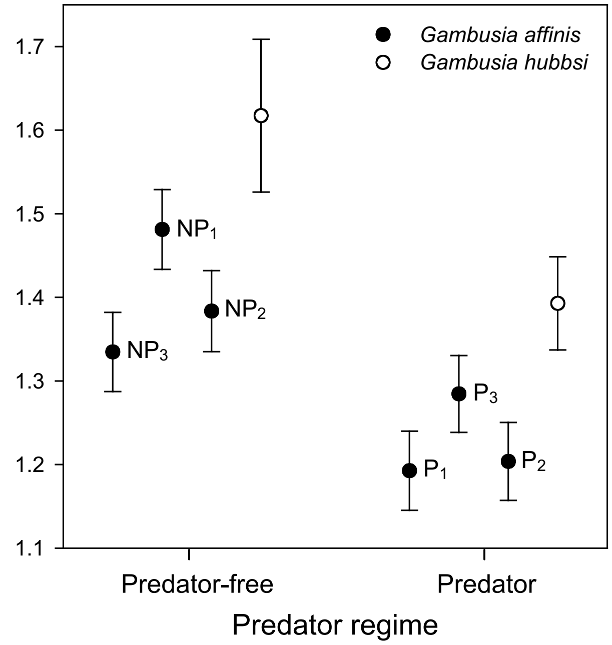
● Gambusia affinis
○ Gambusia hubbsi
1.7
1.6
1.5
1.4
1.3
1.2
1.1
NP1
NP2
NP3
P1
P2
P3
Predator-free
Predator
Predator regime

Figure 5

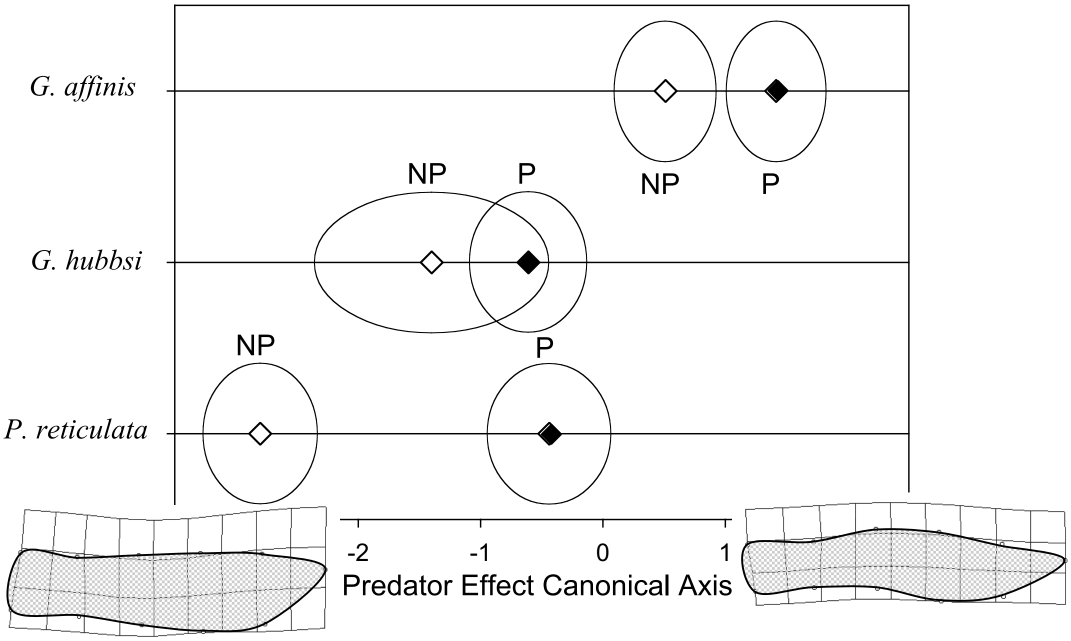
G. affinis
G. hubbsi
P. reticulata
NP
P
NP
P
NP
P
-2
-1
0
1
Predator Effect Canonical Axis

Figure 6

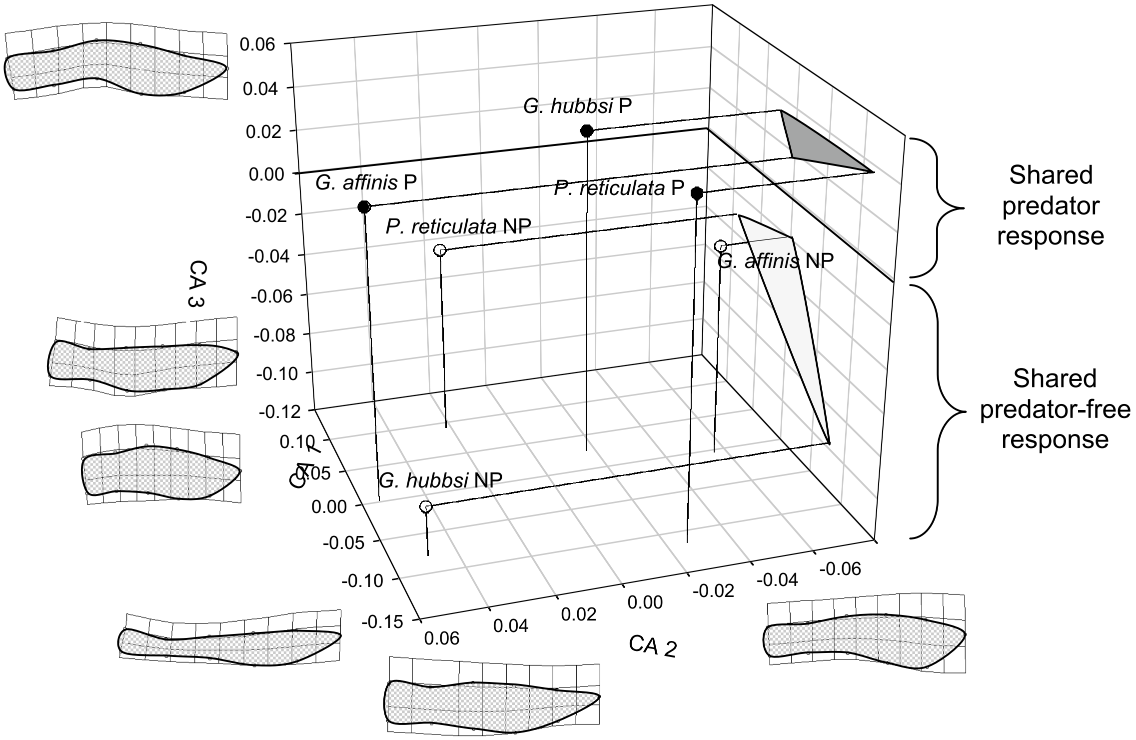
0.06
0.04
0.02
0.00
-0.02
-0.04
-0.06
-0.08
-0.10
-0.12
CA 3
0.10
0.05
0.00
-0.05
-0.10
-0.15
CA 1
0.06
0.04
0.02
0.00
-0.02
-0.04
-0.06
CA 2
G. hubbsi P
G. affinis P
P. reticulata P
P. reticulata NP
G. affinis NP
G. hubbsi NP
Shared predator response
Shared predator-free response

Figure 7