

Trade-off between steady and unsteady swimming underlies predator-driven divergence in *Gambusia affinis*

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Keywords:

adaptation;
biomechanics;
divergent natural selection;
endurance;
geometric morphometrics;
path analysis;
performance trade-off;
population differentiation;
predation;
swimming kinematics.

Abstract

Differences in predation intensity experienced by organisms can lead to divergent natural selection, driving evolutionary change. Western mosquitofish (*Gambusia affinis*) exhibit larger caudal regions and higher burst-swimming capabilities when coexisting with higher densities of predatory fish. It is hypothesized that a trade-off between steady (constant-speed cruising; important for acquiring resources) and unsteady (rapid bursts and turns; important for escaping predators) locomotion, combined with divergent selection on locomotor performance (favouring steady swimming in high-competition scenarios of low-predation environments, but unsteady swimming in high-predation localities) has caused such phenotypic divergence. Here, I found that morphological differences had a strong genetic basis, and low-predation fish required less hydromechanical power during steady swimming, leading to increased endurance. I further found individual-level support for cause-and-effect relationships between morphology, swimming kinematics and endurance. Results indicate that mosquitofish populations inhabiting low-predation environments have evolved increased steady-swimming abilities via stiffer bodies, larger anterior body/head regions, smaller caudal regions and greater three-dimensional streamlining.

Introduction

Divergent natural selection (DNS) – selection pulling trait means of two or more populations toward different adaptive peaks – is generally believed to represent a primary mechanism generating and maintaining phenotypic diversity (e.g. Rice & Hostert, 1993; Orr & Smith, 1998; Schluter, 2000). Although most studies examining DNS have focused on diversification stemming from resource competition, the potential importance of predation as a major driver of evolutionary change has recently attracted considerable attention (e.g. Robinson & Wilson, 1994; Endler, 1995; Smith & Skúlason, 1996; Schluter, 2000; Vamosi, 2005; Langerhans, 2006). Together, these selective agents (competition and predation) form two of the most important sources of natural

selection in the wild. Because the intensity of predation is heterogeneously distributed across space and time, many organisms inhabit both low- and high-predation environments. In most cases, selection via intraspecific competition is predicted to be strongest under the high densities present in low-predation environments – where selection via predation is weakest – and weaken as predation intensity increases and population densities subsequently decline – where selection via predation is strongest (e.g. Hassell, 1975; Holt, 1985; Chesson & Huntly, 1997; Gurevitch *et al.*, 2000; Abrams, 2001; Reznick *et al.*, 2001). Thus, different predation intensities are expected to generate very different selective regimes.

Divergent natural selection typically arises from a combination of (1) a functional trade-off, where organisms cannot simultaneously optimize multiple types of performance, and (2) a shift in the balance of selection on these performance variables across environments, where selection in one environment favours one type of performance but selection in another environment

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favours an opposite type of performance. Both functional trade-offs and environmental heterogeneity are commonplace, and together are presumably largely responsible for much of the phenotypic diversity that exists today. One performance trade-off that is thought to be of general evolutionary importance in fishes is the trade-off between steady and unsteady locomotion (e.g. Lighthill, 1969, 1970; Webb, 1982; Blake, 1983, 2004; Webb, 1984; Videler, 1993; Reidy *et al.*, 2000; Domenici, 2003; Langerhans, 2006, 2008; Langerhans & Reznick, in press). Steady swimming (cruising) describes constant-speed locomotion in a straight line, and is commonly employed in nature during competition for limited resources, such as searching for food (which is often patchily distributed), obtaining mates (sometimes involving long courtship chases) and seeking favourable abiotic conditions (e.g. maintaining preferred environmental temperature) (Plaut, 2001; Domenici, 2003; Blake, 2004). As steady-swimming activities are often of critical importance, natural selection is believed to often favour various means of reducing the energetic cost of movement (i.e. enhance steady-swimming performance). Unsteady swimming refers to more complicated locomotor patterns in which changes in velocity or direction occur, such as fast-starts, rapid turns, braking, and burst-and-coast swimming. In the wild, such activities are common in predator-prey interactions (i.e. capturing evasive prey and evading predatory strikes) and social interactions (e.g. courtship, antagonistic interactions). The most commonly studied form of unsteady swimming is the Mauthner-cell initiated escape response present in most fish, called a 'C-start' (e.g. Weihs, 1973; Eaton *et al.*, 1977; Domenici & Blake, 1997; Hale *et al.*, 2002; Blake, 2004; Domenici, in press). During this fast-start, the fish body rapidly bends into a 'C' shape and then produces a propulsive stroke of the caudal region in the opposite direction, resulting in a sudden, high-energy swimming burst. Thus, fast-start performance is highly important in evading predatory strikes (Webb, 1986a; Katzir & Camhi, 1993; Walker *et al.*, 2005; R.B. Langerhans, unpublished data). These descriptions lead to the hypothesis that selection will favour steady swimming in low-predation environments (to increase competitive abilities), but instead favour unsteady swimming (namely, fast-start performance) in high-predation environments (to increase survival) (e.g. see Walker, 1997; Langerhans *et al.*, 2004, 2007; Langerhans & Reznick, in press).

Traits optimal for steady swimming necessarily compromise unsteady swimming because primary propulsive mechanisms are coupled in most fish (i.e. same structures are used for force generation, transmission and delivery during different swimming modes). This leads to distinctly different phenotype arrangements for optimization of steady and unsteady swimming. Steady swimming is enhanced with a stiff, streamlined body, high proportion of red muscle and a high aspect ratio caudal fin (e.g. Wu, 1971; Lighthill, 1975; Webb, 1975,

1984; Blake, 1983; Weihs, 1989; Videler, 1993; Vogel, 1994; Fisher & Hogan, 2007). These features act to maximize thrust while minimizing drag and recoil energy losses. High unsteady performance is typically produced by a flexible, posteriorly deep body (relatively small head, large caudal peduncle; this might be accomplished by median fins rather than the body), high proportion of white muscle and a large low aspect-ratio caudal fin (e.g. Blake, 1983, 2004; Webb, 1983, 1984, 1986b; Walker, 1997; Langerhans *et al.*, 2004). These features maximize thrust and stability during rapid bouts of swimming activity. Using these biomechanical relationships, combined with the hypothesis of DNS for steady and unsteady swimming between predator regimes, we can make specific predictions for the evolution of phenotypes in different predatory environments: stiffer, more streamlined bodies, higher aspect-ratio caudal fin, higher ratio of red : white muscle, higher steady-swimming performance and lower unsteady-swimming performance in fish inhabiting low-predation environments compared with fish in high-predation environments (see Langerhans & Reznick, in press).

Some of the strongest evidence to date for the role of predation in driving phenotypic evolution in fish comes from livebearing fishes (Family Poeciliidae, e.g. Reznick & Endler, 1982; Endler, 1995; Reznick *et al.*, 1997; Johnson & Belk, 2001; Jennions & Telford, 2002; O'Steen *et al.*, 2002; Ghalambor *et al.*, 2004; Langerhans & DeWitt, 2004; Langerhans *et al.*, 2005, 2007; Langerhans & Reznick, in press). For instance, populations of western mosquitofish (*Gambusia affinis*) coexisting with large, predatory fish exhibit smaller anterior body/head regions, larger caudal regions and higher unsteady (burst swimming) locomotor performance than conspecific populations inhabiting waters lacking piscivorous fish (Langerhans & DeWitt, 2004; Langerhans *et al.*, 2004). This system, however, still harbours a number of important, unanswered questions: (1) Have these differences arisen as a response to DNS for steady and unsteady locomotion between predator regimes? Here I address this question by testing whether low-predation populations—which have reduced burst-swimming performance (Langerhans *et al.*, 2004) – exhibit greater steady-swimming performance relative to high-predation populations. (2) Are differences in morphology and swimming performance due to genetically based differentiation? In this study, I test for a genetic basis to phenotypic divergence by examining first- and second-generation adult males and females that were born and raised in a common laboratory environment. (3) Has divergence between predator regimes manifested as a result of individual-level, cause-and-effect relationships between morphology and swimming performance? Here I address the mechanistic basis of phenotypic divergence by investigating the interrelationships among morphology, steady-swimming kinematics and endurance using path analysis.

Methods

Study system and experimental animals

G. affinis is a widespread, livebearing fish that occupies a broad range of habitats across the south-central United States and north-eastern Mexico (Meffe & Snelson, 1989). These small fish (maximum ~50 mm standard length) are a common prey item for many coexisting piscivorous fish, and are known to exhibit phenotypic differences between low- and high-predation environments (Langerhans & DeWitt, 2004; Langerhans *et al.*, 2004, 2005). In the face of high levels of predation, the importance of unsteady-swimming activities is straightforward: increased acceleration and velocity during predator evasion should increase survival. In the absence of predators, steady-swimming performance is predicted to be of critical ecological importance, as *Gambusia* populations are often found at high densities in these localities (suggesting higher levels of intraspecific competition), and make greater use of open-water habitat (an area too dangerous to utilize in high frequency in high-predation environments) where fish continuously swim in search of prey patches and mating opportunities (Horwood & Cushing, 1977; Winkelman & Aho, 1993; Langerhans, 2006; R.B. Langerhans, unpublished). Thus, the natural history of this system appears consistent with the hypothesis of DNS between predator regimes on locomotor performance.

G. affinis individuals examined in this study were derived from fish originally collected from six freshwater ponds in Brazos County, Texas, in March 2003: three populations coexisted with large, predatory fish (e.g. *Micropterus salmoides*, *Lepomis cyanellus*, *Pomoxis annularis*), whereas three populations did not coexist with any piscivorous fish (see further descriptions in Langerhans *et al.*, 2004, 2005). Predatory fish were surveyed on numerous occasions using seines, cast nets, dip nets, and hook-and-line angling to ensure that predator communities were accurately assessed. These populations are relatively isolated from one another, but are geographically proximate (straight-line geographical distance; range: 0.80–17.34 km), with similar distances between populations of either the same (mean \pm 1 SE; 10.32 ± 2.34 km) or different predator regime (8.17 ± 1.93 km).

Wild-caught females were held in the laboratory for approximately 1 month before delivering offspring in an attempt to reduce potential maternal effects associated with natal environments. First-generation (F1) offspring were acquired from wild-caught females (six females from each population) and raised in 15-L aquaria (two tanks per population). Second-generation (F2) offspring were acquired from F1 females (two females from each population) and raised in 30-L aquaria (one tank per population). Fish densities were similar for all populations (mean \pm SE, 0.27 ± 0.03 fish per litre), and did not differ between predator regimes (*t*-test, $P = 0.45$). All fish

were reared with a 14L : 10D photoperiod. Fish were fed newly hatched brine shrimp nauplii daily until 16 weeks of age when fish began receiving frozen daphnia and bloodworms. To reduce the possible effects of microenvironmental factors, aquaria were arranged side by side in the laboratory, alternating between low- and high-predation populations.

Morphometrics

To assess lateral body shape, I digitized 10 landmarks (Fig. 1a) on digital images of each specimen ($n = 83$; 54 F1, 29 F2) using TPSDIG (Rohlf, 2006), and used geometric morphometric methods to examine morphological variation (Rohlf & Marcus, 1993; Marcus *et al.*, 1996; Zelditch *et al.*, 2004). For further description of landmarks and methods, see Langerhans *et al.* (2004), Langerhans & DeWitt (2004) and Langerhans *et al.* (2007). I used TPSREG software (Rohlf, 2005) to perform generalized least-squares Procrustes superimposition (i.e. align landmark coordinates by rotating, translating and scaling coordinates to remove positioning effects and isometric size effects; Bookstein, 1991; Marcus *et al.*, 1996) and obtain shape variables (uniform components and partial warps) for analysis.

In addition to lateral body shape, I was interested in variation in three-dimensional streamlining. For fish bodies (and airship hulls), streamlining is often discussed relative to volume (as fish must carry internal organs and muscles, and cannot be two-dimensional), where a streamlined rigid body is not simply elongate, but rather exhibits a fusiform shape that minimizes drag while maximizing volume (i.e. approximating the volume distribution of a foil; von Mises, 1945; Hoerner, 1965; Blake, 1983; Weihs & Webb, 1983; McHenry & Lauder,

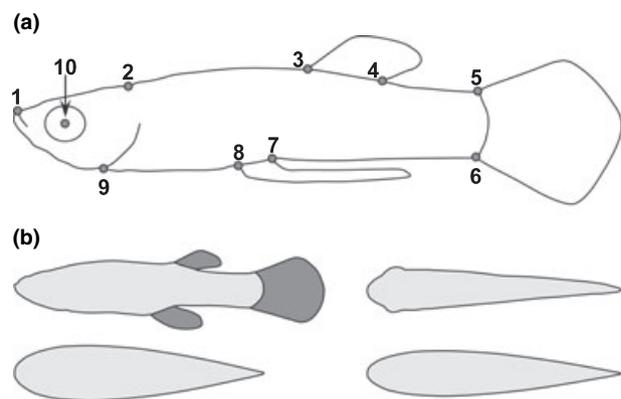


Fig. 1 Illustration of morphometric methods. (a) Landmarks used for examination of lateral body shape (male depicted). (b) Body outlines (upper panel; female depicted) from both lateral (left) and ventral (right) perspectives were used to compare volume distribution between fish bodies and streamlined foils (lower panel) for assessment of three-dimensional streamlining.

2006). Thus, a streamlined shape exhibits an anteriorly positioned (~30% of total length from nose) maximum thickness (diameter ~22% of total length) and tapers to a small posterior end (excluding the tail; see Fig. 1b). To evaluate three-dimensional streamlining, I calculated a recently described streamlining index (SI; for details, see Langerhans & Reznick, in press) for each fish used in the steady-swimming performance experiment ($n = 45$; 20 F1, 25 F2). This index represents a slight modification of the streamlining ratio described by McHenry & Lauder (2006), in which the volume distribution of a fish's body is compared to a body having the profile of a streamlined shape (foil drawn from the U.S. National Advisory Committee for Aeronautics, NACA). I used lateral and ventral images of specimens to compare a fish's volume distribution to that of a NACA-streamlined body (Fig. 1b). The SI can range from $-\infty$ to 1.0, with 1.0 representing a perfect match in volume distribution between a fish's body and the NACA-streamlined body (see Appendix S1 for details).

Steady-swimming performance

I investigated steady-swimming performance of laboratory-reared *G. affinis* by examining morphology, swimming kinematics and endurance (see Table 1) during constant-velocity swimming in a swim chamber. All fish were starved 24 h prior to experimentation to ensure a post-absorptive state (Niimi & Beamish, 1974). Swimming trials were conducted in a 40-L flow tank holding a plexiglass chamber with a working section of $10 \times 60 \times 10$ cm. A propeller pump (Leader Provort 540a; Ladson, SC, USA) generated flow into the chamber, and water temperature was maintained at approximately 27 °C (27.4 ± 0.38 °C) using ice packs placed near the pump motor. A straw grid at the upstream end of the working section smoothed water flow within the chamber and a mesh screen at the downstream end prevented fish from escaping.

Each fish was tested at a flow speed of 0.20 m s^{-1} , measured with a velocity probe and meter (Flow Probe

101; Global Water, Gold River, CA, USA). This speed was selected in an attempt to provide ecologically meaningful estimates of steady-swimming performance and to ensure that I examined *prolonged* swimming, the swimming category between burst and sustained swimming, where speeds can be maintained for 20 s to 200 min (Beamish, 1978). The flow speed used here provides a moderately fast swimming speed for *G. affinis* ($\sim 5\text{--}8 L \text{ s}^{-1}$, where L is total body length), corresponding to speeds commonly employed in the wild during activities such as antagonistic foraging interactions and inter-sexual chases (personal observation). Because all fish examined here primarily experienced inertial forces at the high Reynolds numbers (Re) achieved during steady-swimming trials (Re of 5000–11 000), standard hydrodynamic theories of undulatory swimming (e.g. rigid- and elongated-body theories) can be appropriately applied to these fish (e.g. see Pedley & Hill, 1999; McHenry & Lauder, 2006). A pilot study confirmed that individuals typically fatigued within 4 min at this flow speed, yielding fatigue times in the range of those commonly reported in studies of endurance (e.g. Videler & Wardle, 1991; Nikora *et al.*, 2003; Blake *et al.*, 2005). This experimental design should primarily assess aerobic-swimming performance (e.g. require minimal recruitment of white muscle fibres). To this end, measurement of swimming kinematics (see below) during steady swimming should provide a largely exclusive examination of aerobic-swimming performance, whereas the estimation of overall endurance (see below) may inevitably involve some degree of anaerobic-swimming performance (a fact common to all studies involving fatigue).

To measure kinematic variables, all trials were recorded from above with a high-speed digital video camera (Fastec TroubleShooter HR; San Diego, CA, USA) set to 125 frames s^{-1} and 640×480 pixel resolution. A 2-cm grid was affixed underneath the aquarium glass for scale, and a 45° mirror permitted the simultaneous observation of dorsal and lateral aspects of the fish. I selected five kinematic variables for measurement based on their potential importance in the hydrodynamics of steady swimming (see Table 1). Assuming a fairly simplistic model of undulatory swimming, where fish swimming is modelled as an actuator-driven, flexible body (see fig. 11 in McHenry *et al.*, 1995), swimming speed can be controlled by modifying three variables: body stiffness (estimated here as propulsive wavelength, λ), driving frequency (estimated here as tail-beat frequency, f) and driving amplitude (estimated here as rostral amplitude, R). Together, these three parameters determine the propulsive wave speed (calculated here as $c = \lambda f$) and tail-beat amplitude (H , measured from video sequences here) – consequently determining swimming speed (U , held constant here at 0.20 m s^{-1}). If fish from high-predation environments suffer reduced steady-swimming abilities compared with low-predation fish as predicted, then this should be reflected by differences in at least one of these parameters. Because

Table 1 Variables measured or calculated for all fish examined in the steady-swimming performance experiment.

Data type	Variable
Morphology	Body size
	Lateral body shape
	3D streamlining
Kinematics	Caudal fin depth
	Tail-beat frequency
	Rostral amplitude
	Tail-beat amplitude
	Propulsive wavelength
Force production	Propulsive wave speed
	Power
Endurance	Fatigue time

steady-swimming performance is enhanced with stiffer bodies, while fast-start performance is enhanced with more flexible bodies (Webb, 1984; Videler, 1993; Long & Nipper, 1996; Pabst, 1996; Brainerd & Patek, 1998; Dickinson *et al.*, 2000; Domenici, 2003; Blake, 2004), low-predation fish are predicted to exhibit greater flexural stiffness than fish from high-predation environments. If high-predation fish do exhibit lower body stiffness, they should compensate for this deficiency during steady swimming by either increasing driving frequency, driving amplitude or both. Furthermore, such modifications to steady-swimming kinematics are expected to lead to greater hydromechanical work produced by high-predation fish relative to low-predation fish swimming at the same speed. To provide an overall summary of the magnitude of thrust production, I estimated total hydromechanical power (P). Appendix S1 provides a detailed description of how each of these variables was measured.

Endurance was estimated for each fish as the time to fatigue (F_t), defined as the time from initiation of flow until the fish ceased swimming and fell back against the downstream screen. This measurement is meant to provide a summary metric of organism-level endurance, which is assumed to provide a strong estimate of fitness in low-predation environments, where fish are continually cruising throughout the environment due to the lack of predation risk and high intensity of competition.

Because endurance and several kinematic variables are expected to scale with body size, I measured total body length (L , mm) of each fish, and included this variable in all analyses. Other measurements of size (mass, surface area) were highly correlated with body length (both $r > 0.98$, $P < 0.0001$), and produced very similar results.

Statistical analysis

Differences in overall lateral body shape between predator regimes were tested using nested multivariate analysis of covariance (MANCOVA), where geometric shape variables (uniform components and partial warps) served as dependent variables, centroid size served as the covariate (controlling for multivariate allometry), and predator regime and population nested within predator regime served as independent variables. Centroid size – the typical estimate of body size in geometric morphometric studies – is the square root of the summed, squared distances of all landmarks from their centroid. Analyses were initially conducted separately for F1 and F2 generations. Terms for sex and the interaction between sex and predator regime were initially included in both models, and nonsignificant terms were removed for final analyses. In both cases, the term for sex was retained whereas the interaction term was removed (nonsignificant for F1 and F2 fish; $P = 0.90$ and 0.68 respectively). This indicated that sexes differed in morphology, but both males and females exhibited a similar

nature of morphological differentiation between predator regimes. Because these models revealed highly similar results among laboratory-reared generations, I only present here the final analysis where fish were pooled across generations to provide an overall test of the genetic basis of morphological differences between predator regimes. Again, a term for sex, but not the interaction between sex and predator regime ($P = 0.29$), was retained in this analysis. Heterogeneity of slopes (interaction between centroid size and predator regime) was nonsignificant in all models, and thus excluded from the final analysis.

To determine the nature of morphological divergence between predator regimes, I performed a canonical analysis of the predator regime term in MANCOVA. Rather than deriving a standard canonical variate from the predator regime term [i.e. principal components analysis (PCA) of $\mathbf{E}^{-1}\mathbf{H}$, where \mathbf{E}^{-1} is the inverse of the error sums of squares and cross-products matrix, and \mathbf{H} is the sums of squares and cross-products matrix of the term of interest], I wished to avoid scaling the multidimensional space by a matrix inverse. Such scaling prior to matrix diagonalization generates some degree of distortion in the multivariate space relative to the original shape space unless within-group variation is isotropic (i.e. $\mathbf{E} \propto$ identity matrix), which is virtually never the case with landmark data (see Klingenberg & Monteiro, 2005). Thus, I performed diagonalization of \mathbf{H} directly. That is, I performed a PCA of \mathbf{H} (for the predator regime term) to derive an eigenvector of divergence (\mathbf{d}) in body shape between predator regimes. \mathbf{d} describes the linear combination of dependent variables exhibiting the greatest difference between predator regimes in Euclidean space.

Random nested factors are not often possible in a MANOVA framework (matrix determinants are negative, and thus the term is un-testable, if the error degrees of freedom does not equal or exceed the number of dependent variables), forcing the use of only fixed-effects which can cause the test of the main effect to exhibit inflated type I error rates if the nested term is significant. Because the nested term was significant here, I conducted mixed-model nested analysis of covariance (ANCOVA) using \mathbf{d} as the dependent variable. This model was identical to the nested MANCOVA with the exceptions that the divergence vector was the sole dependent variable and population nested within predator regime was designated a random factor. Simulations indicate that the nested ANCOVA performed here with \mathbf{d} only yields significant results when the underlying dependent variables truly exhibit differences between groups (R.B. Langerhans, unpublished data).

Shape variation along divergence vectors (\mathbf{d}) was visualized using thin-plate spline transformation grids (for details, see Bookstein, 1991; Rohlf *et al.*, 1996; Klingenberg *et al.*, 2003; Klingenberg & Monteiro, 2005), and additionally examined using Pearson correlation coefficients between landmark positions and

divergence vectors. To evaluate the overall magnitude of shape differences between populations, I calculated Procrustes distance, the standard metric for shape dissimilarity in geometric morphometrics (Bookstein, 1996; Dryden & Mardia, 1998). Procrustes distance was calculated as the Euclidean distance between superimposed shapes. I obtained means and standard errors of Procrustes distances between predator regimes using 1000 bootstraps of pairwise Procrustes distances between populations.

Following common procedure, and to allow results to be comparable with previous studies, I transformed the following parameters by dividing them by body length prior to analysis: caudal fin depth, rostral amplitude, tail-beat amplitude, propulsive wavelength and propulsive wave speed. Because these 'size-adjusted' variables might still exhibit scaling, I further included body size as a covariate in all analyses (see below). As results are nearly identical if log-transformed lengths are used instead of ratios, I only present results using the ratios. All data were inspected to meet assumptions of normality for parametric statistical models, and the following transformations were made: arcsin square-root of SI, and \log_{10} of body length, power and fatigue time.

For fish used in the steady-swimming performance experiment, I first conducted an overall test of differences in swimming kinematics between predator regimes using nested MANCOVA as described above. In this case, I used the five kinematic variables (see Table 1) as dependent variables, and body length served as the covariate. Because the nested term was not significant, I did not perform an additional mixed-model nested ANCOVA with the divergence vector (**d**). Following a significant MANCOVA, I then conducted nested ANOVA for body length, and nested ANCOVA for all other response variables to test for differences between predator regimes. For each model, I tested for effects of predator regime and population nested within predator regime (random factor), controlling for effects of body size. Sex, generation (F1 or F2), and their interactions were initially included in all models and nonsignificant terms were removed from the final analyses. The generation term and interactions involving the generation term were never significant, the sex term was only retained for the model of body length, and the sex and sex \times predator regime terms were included only for the analysis of hydromechanical power. Heterogeneity of slopes was nonsignificant in all cases, and thus excluded from final models. To aid in the interpretation of the magnitude of differences between predator regimes I present standardized effect sizes (Cohen's *d*, describing the difference between means in standard deviation units; Cohen, 1988) along with 95% confidence intervals (calculated using non-central *t* distributions; e.g. Venables, 1975; Cumming & Finch, 2001; Fidler & Thompson, 2001; Smithson, 2003; Zou, 2007).

To examine relationships among morphology, swimming kinematics and endurance, I conducted a path analysis (e.g. Wright, 1934; Mitchell, 1992, 2001; Shipley, 1997; Kline, 2005). This analysis was meant to provide a more detailed and multivariate understanding of the causes of variation in swimming kinematics and endurance, as well as the manner in which locomotor performance has evolved in different predator regimes. I assumed a directionality for causality in the analysis, where morphology might influence swimming kinematics (but not vice versa), and both morphology and swimming kinematics might affect endurance (but not vice versa). The opportunity for direct effects of morphology on endurance was included in the analysis because of the possible existence of unmeasured performance variables (Garland & Losos, 1994; Johnson *et al.*, 2008) and to allow for scaling with size. To reduce dimensionality and multicollinearity, I conducted PCA using correlation matrices for the four morphological variables and the five kinematic variables (see Table 1). Thus, PC axes were constructed separately for morphology and kinematics, and subsequently used in path analysis. I retained all PC axes that explained more variation than that expected under a broken-stick model (Frontier, 1976; Jackson, 1993). I evaluated the overall fit of the path model using the Bollen–Stine bootstrap test (Bollen & Stine, 1992), which tests the null hypothesis that the *a priori* designation of paths is correct. All path coefficients were estimated using maximum likelihood, and significance was assessed using the critical ratio ($z = \text{coefficient divided by its standard error}$), which has a standard normal distribution under parametric assumptions. Path analysis was conducted with AMOS version 5.0.1 (Arbuckle & Wothke, 1999; Arbuckle, 2003).

It is possible for this analysis to be confounded by differences between predator regimes. For example, the path analysis could suggest that swimming kinematics influenced variation in endurance, whereas in reality the two factors might simply covary with predator regime, leaving the true causal mechanism a mystery. In an effort to alleviate this concern, and assess the consistency of results among predator regimes, I conducted the path analysis within each predator regime and combined probabilities (*P*-values for path coefficients) using a weighted *Z*-transform test (also known as Stouffer's method; Whitlock, 2005). The reciprocal of the squared standard error was used to weight each test (see Whitlock, 2005). The idea underlying such an approach is that stronger evidence for cause-and-effect relationships is garnered when the associations are consistent within both low- and high-predation environments (e.g. Langerhans *et al.*, 2004, 2007). I could not conduct an additional weighted *Z*-transform test to combine probabilities across analyses performed within each population because degrees of freedom were exhausted within two of the six populations due to low sample sizes. However,

results from a weighted Z-transform test using the remaining four populations produced qualitatively similar results as the analysis described above. This suggests that the findings are robust across both within-predator regime analysis and within-population analysis. Because *a priori* directional hypotheses exist for several analyses (e.g. higher endurance in low-predation populations), one-tailed significance values may be used. All *P*-values presented, however, are two-tailed unless otherwise indicated.

Results

Lateral body shape

Lateral body shape significantly differed between predator regimes (Table 2; Fig. 2). Although the magnitude of divergence appears slightly greater for F1 fish than F2 fish, this was not significant, as a term for the interaction between rearing generation and predator regime was nonsignificant when included in the models (MANCOVA: $P = 0.22$; ANCOVA: $P = 0.27$). Interpretation of the nature of morphological differences using correlations between superimposed landmark coordinates and the divergence vectors (**d**) derived from the predator regime term in MANCOVA (Table 3), as well as using thin-plate spline transformation grids (Fig. 3), revealed that body shape divergence in laboratory-reared fish is highly similar to that observed in wild-caught fish. Specifically, laboratory-reared fish derived from high-predation populations exhibited smaller anterior body/head regions, larger caudal regions, and a relatively posterior placement of the eye compared with fish derived from low-predation populations. Moreover, based on Procrustes distances between populations, the overall magnitude of morphological divergence observed in the wild (mean \pm 1 SE; females: 0.030 ± 0.001 ; males: 0.034 ± 0.004 ; data from Langerhans *et al.*, 2004) was similar to that observed in first-generation (females: 0.035 ± 0.005 ; males: 0.039 ± 0.003) and second-generation (females: 0.031 ± 0.004 ; males: 0.037 ± 0.003) laboratory-born fish raised in a common environment. These results suggest that

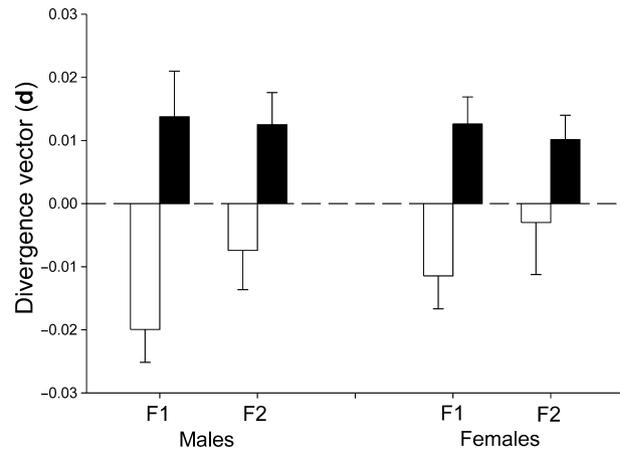


Fig. 2 Genetic basis of morphological divergence between low-predation (open bars) and high-predation (filled bars) populations of *Gambusia affinis* as depicted by the divergence vector (**d**) derived from MANCOVA (mean \pm 1 SE).

morphological differences between predator regimes largely reflect genetically based differentiation.

Steady-swimming performance

MANCOVA revealed significant differences between predator regimes in steady-swimming kinematics ($F_{5,34} = 10.99$, $P < 0.0001$), whereas body length was marginally significant ($F_{5,34} = 2.15$, $P = 0.08$), and population nested within predator regime was nonsignificant ($F_{20,113.7} = 0.85$, $P = 0.65$). Inspection of **d** suggested that high-predation fish exhibited higher rostral and tail-beat amplitudes, but smaller propulsive wavelengths and wave speeds than low-predation fish (with no difference in tail-beat frequency) (Table S1). Univariate tests found no evidence for differences between predator regimes in body length, caudal fin depth or tail-beat frequency, but differences were either suggestive or evident for all other variables (Table 4) – results consistent with MANCOVA. Although differences between predator regimes in

Table 2 Nested multivariate analysis of covariance (MANCOVA) examining overall lateral body shape (uniform components and partial warps), and nested univariate analysis of covariance (ANCOVA) examining lateral body shape variation described by the divergence vector (**d**). The population nested term was designated a fixed effect in the MANCOVA (see text) and a random effect in the ANCOVA. *F*-ratios were approximated using Wilks's Λ values for the population nested within predator regime term in MANCOVA.

Source	MANCOVA			ANCOVA		
	<i>F</i>	d.f.	<i>P</i>	<i>F</i>	d.f.	<i>P</i>
Centroid size	7.95	16, 60	< 0.0001	0.13	1, 75	0.7225
Predator regime	3.55	16, 60	0.0002	13.71	1, 4.17	0.0193
Population (predator regime)	2.03	64, 237.17	< 0.0001	2.83	4, 75	0.0305
Sex	35.63	16, 60	< 0.0001	23.16	1, 75	< 0.0001

Table 3 Pearson correlation coefficients between superimposed landmark coordinates and the divergence vectors (**d**) derived from the predator regime term of MANCOVAs for laboratory-reared and wild-caught *Gambusia affinis* (values for wild-caught fish reflect averages for males and females from data in Langerhans *et al.*, 2004). Landmark numbers follow Fig. 1. Directionality of the shift in landmark positions is presented for high-predation populations, relative to low-predation populations (e.g. landmark 1 is relatively posterior in high-predation populations). Variables exhibiting a correlation ≥ 0.4 are in bold text.

Landmark	Laboratory-reared	Wild-caught	Direction
1X	+0.77	+0.67	Posterior
1Y	-0.19	+0.05	-
2X	+0.11	-0.16	-
2Y	-0.71	-0.46	Ventral
3X	-0.36	-0.21	-
3Y	+0.27	-0.07	-
4X	-0.38	-0.13	-
4Y	+0.48	+0.06	Dorsal
5X	+0.67	+0.61	Posterior
5Y	+0.12	+0.20	-
6X	+0.57	+0.62	Posterior
6Y	-0.08	+0.14	-
7X	-0.54	-0.88	Anterior
7Y	-0.19	-0.26	-
8X	-0.48	-0.78	Anterior
8Y	-0.18	+0.03	-
9X	-0.01	+0.21	-
9Y	+0.48	+0.45	Dorsal
10X	+0.60	+0.62	Posterior
10Y	-0.18	-0.11	-

streamlining were not statistically significant using an α of 0.05 – apparently due to one low-predation population exhibiting relatively nonstreamlined bodies (see Fig. 4) – I interpret these results as strongly suggestive, as the direction of differences matched *a priori* predictions (i.e. one-tailed $P = 0.034$), and the effect size was fairly large with little overlap of the 95% confidence interval beyond zero. For traits associated with body length, larger fish had greater streamlining, lower tail-beat frequency and propulsive wave speed, and higher power and fatigue time. Relative to low-predation populations (controlling for body size), fish derived from high-predation populations tended to exhibit lower streamlining, higher rostral and tail-beat amplitudes, lower propulsive wavelengths, lower propulsive wave speeds, higher power and lower fatigue times (Fig. 4). These results indicate that fish derived from different predator regimes swam in distinctly different manners. Swimming at the same speed, fish from low-predation populations produced thrust in a more energetically efficient manner by employing low-amplitude and long-wavelength undulations (Fig. 4; visualized in Fig. 5). This manner of swimming resulted in lower overall power consumption at a given swimming speed, and consequently higher endurance for fish derived from low-predation populations.

Path analysis

Using PCA to eliminate redundant dimensions and provide a more multivariate perspective to the relationships among morphology, swimming kinematics and endurance, I retained the first two PC axes for morphological variables and the first three PC axes for kinematic variables (Table S2). Only these PC axes explained more variation than expected under a broken-stick model, and accounted for 63.18% and 95.48% of the variance in the morphological and kinematic data sets respectively. I constructed a path model as described above using these five PC axes and the estimate for endurance ($\log_{10} F_t$). PCA effectively reduced dimensionality (five PC axes rather than nine measured variables), eliminated multicollinearity for the paths between morphology and swimming kinematics, and reduced multicollinearity for all other paths (all variance inflation factors < 1.30). There was no indication that the path model was inadequately structured (Bollen–Stine bootstrap, $P = 0.98$).

I found evidence for five significant path coefficients (Fig. 6). These coefficients described the following relationships: (1) fish with larger anterior body/head regions and smaller caudal regions produced smaller amplitude undulations, but higher wave speeds, (2) larger, more streamlined fish swam with lower tail-beat frequencies, (3) fish with larger anterior body/head regions and smaller caudal regions swam with lower tail-beat frequencies, (4) fish with lower rostral and tail-beat amplitudes and higher wave speeds had higher endurance, and (5) larger, more streamlined fish exhibited higher endurance.

Because theory predicts that fish with larger anterior body/head regions and smaller caudal regions should exhibit higher endurance via reduced drag (and this is the major difference in morphology between predator regimes), I tested for an indirect effect of $PC2_{Morph}$ on endurance. This was accomplished by constructing a one-sided bootstrapped confidence interval for the estimated indirect effect using 10 000 bootstraps of the data set. I found that $PC2_{Morph}$ did have a marginally significant indirect effect on endurance via its direct effects on $PC1_{Kine}$ (standardized indirect coefficient \pm SE, 0.10 ± 0.08 , one-tailed $P = 0.079$). That is, fish with larger heads and smaller caudal peduncles tended to exhibit higher endurance because those fish also produced low amplitude undulations with high wave speeds (i.e. utilized a more energetically efficient manner of thrust production).

Using the weighted Z-transform method, I found that four of the five significant path coefficients tended to be consistent across predator regimes: $PC1_{Morph}$ to $PC3_{Kine}$ (one-tailed $P = 0.001$), $PC1_{Morph}$ to endurance (one-tailed $P = 0.0004$), $PC2_{Morph}$ to $PC1_{Kine}$ (one-tailed $P = 0.081$) and $PC1_{Kine}$ to endurance (one-tailed $P = 0.013$). In all these cases, the direction of the trend

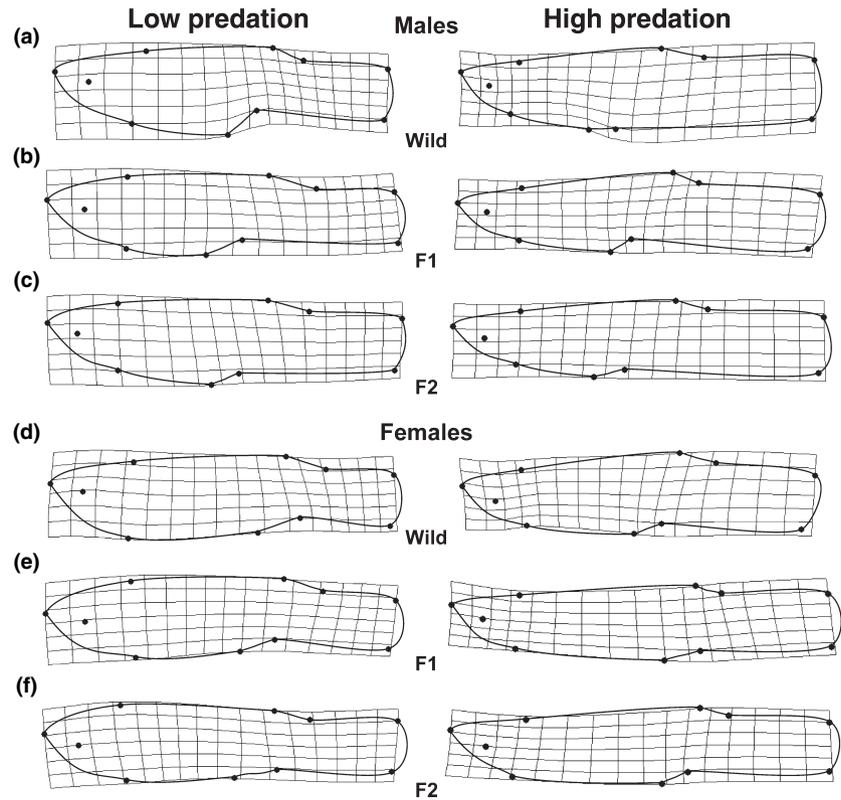


Fig. 3 Morphological divergence between low-predation (left) and high-predation (right) populations of *Gambusia affinis*: (a) wild-caught males, (b) first-generation laboratory-reared males, (c) second-generation laboratory-reared males, (d) wild-caught females, (e) first-generation laboratory-reared females, (f) second-generation laboratory-reared females. Body shape differences (i.e. variation described by divergence vectors, **d**, derived from MANCOVA) are illustrated using thin-plate spline transformation grids relative to mean landmark positions (magnified $2 \times$ in all cases to better demonstrate differences). Solid lines connecting outer landmarks are drawn to aid interpretation. Note the larger anterior body/head region and smaller caudal peduncle in low-predation populations. Data for wild-caught fish from Langerhans *et al.* (2004).

Table 4 Nested analysis of variance (ANOVA) and analysis of covariance (ANCOVA) results examining variation in morphology, kinematics and endurance among *Gambusia affinis* populations. Cohen's *d* is the standardized effect size for the predator regime term, and numbers in parentheses represent the lower and upper bounds of its 95% confidence interval.

Dependent variable	R^2	Cohen's <i>d</i>	Predator regime <i>P</i>	Population (predator regime) <i>P</i>	Body length <i>P</i>
Body length ($\log_{10} L$)*	0.18	0.07 (−0.54, 0.67)	0.7986	0.5385	
Streamlining index ($\arcsin \sqrt{SI}$)	0.23	0.69 (−0.05, 1.37)	0.0683	0.8083	0.0155
Caudal fin depth (B/L)	0.34	0.08 (−0.54, 0.67)	0.8137	0.0035	0.5141
Tail-beat frequency (<i>f</i>)	0.22	0.22 (−0.41, 0.82)	0.5093	0.6673	0.0076
Rostral amplitude (R/L)	0.33	1.22 (0.24, 2.14)	0.0116	0.4817	0.3012
Tail-beat amplitude (H/L)	0.43	1.19 (0.22, 2.14)	0.0152	0.1968	0.7596
Propulsive wavelength (λ/L)	0.29	2.17 (0.95, 3.33)	0.0001	0.9226	0.7267
Propulsive wave speed (c/L)	0.29	0.80 (0.00, 1.52)	0.0502	0.6207	0.0103
Power ($\log_{10} P$)†	0.68	0.89 (0.22, 1.53)	0.0112	0.5899	< 0.0001
Fatigue time ($\log_{10} F_t$)	0.19	0.84 (0.07, 1.55)	0.0320	0.8760	0.0476

*Model also included a term for sex.

†Model also included terms for sex and sex \times predator regime.

was the same within each predator regime. However, the path from $PC2_{\text{Morph}}$ to $PC3_{\text{Kine}}$ was not consistent across predator regimes (one-tailed $P = 0.435$). Moreover, combining results obtained within four of the six populations (only four populations had enough degrees of freedom to perform the path analysis) concurred with these results.

Discussion

Although a number of factors might influence divergent selection between low- and high-predation environ-

ments (e.g. sexual selection, diet), a major prediction involves selection on locomotor capabilities arising from differences in the relative importance of competition and predation. Results of this study are consistent with the hypothesis of DNS between predator regimes for steady and unsteady locomotion. The findings demonstrate a genetic basis to phenotypic differences between predator regimes in *G. affinis*, and reveal that phenotypic divergence goes far beyond the previously documented cases of lateral body shape and burst-swimming abilities. Fish derived from low-predation environments also tended to

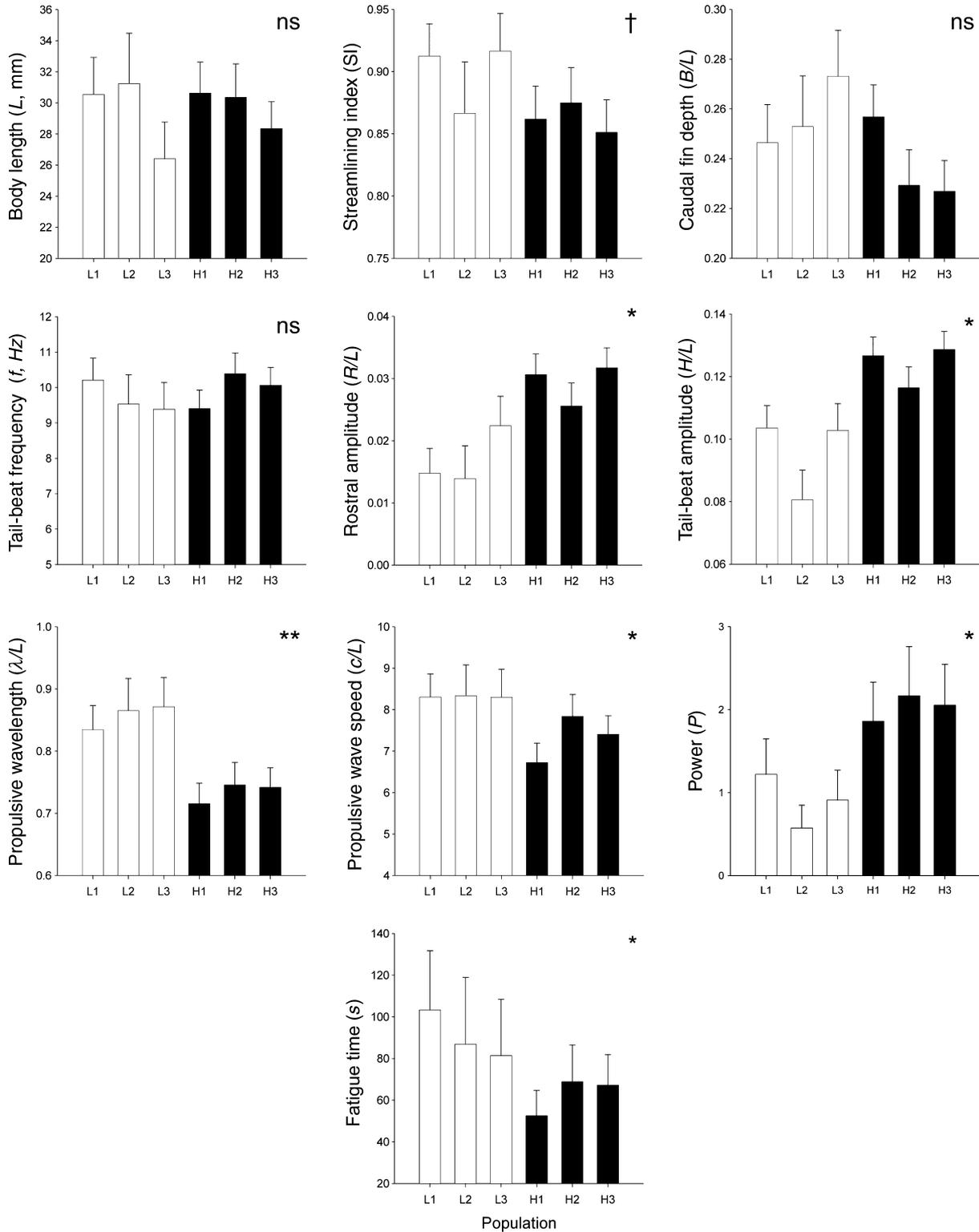


Fig. 4 Variation in morphology, kinematics and endurance among *Gambusia affinis* populations (least-squares means \pm 1 SE). Open bars represent low-predation populations and filled bars represent high-predation populations. All values control for possible effects of body size, and are given for the average body length of 29.59 mm (slopes were homogeneous in all cases). Significant differences between predator regimes are denoted in the upper-right corner of each graph: ns, nonsignificant; † $P < 0.10$; * $P \leq 0.05$; ** $P < 0.001$.



Fig. 5 Visualization of swimming kinematics during a complete tail beat for *Gambusia affinis* individuals derived from low- and high-predation environments. Amplitude envelope of body undulations depicted by midlines (as viewed from above), where x -positions of the snout were made to coincide ($\Delta t = 0.008$ s; snout tip on left, tail tip on right). Fish from low-predation populations generate a long body wave with a narrow amplitude envelope compared with high-predation fish. Midlines were drawn from representative individuals either near, or more extreme than, the mean of measured kinematic variables for each predator regime (depicted are females derived from L3 and H2; these two populations are only approximately 800 m apart).

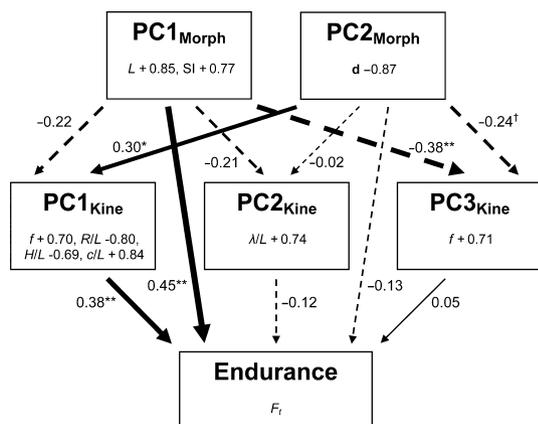


Fig. 6 Path analysis results examining relationships among morphology, swimming kinematics and endurance. Numbers beside arrows indicate standardized path coefficients, and line thickness reflects the strength of the path. Solid lines represent positive effects and dashed lines represent negative effects. Primary factor loadings (i.e. loadings ≥ 0.61) for each principal component are given inside its respective box. Trait abbreviations follow Table 4 and Fig. 4. Morph, morphology data set; Kine, kinematics data set; $^\dagger P < 0.10$; $*P < 0.05$; $**P < 0.01$.

exhibit greater three-dimensional streamlining, employ a more energetically efficient means of thrust production during steady swimming and have higher endurance than fish derived from high-predation localities. Using a multivariate path analytic approach, I also found evidence consistent with cause-and-effect relationships predicted by theory, and implicated in divergent natural selection and subsequent predator-driven evolutionary divergence in this system.

Lateral body shape

Although previous evidence suggests that morphological divergence in *G. affinis* has a genetic basis (Langerhans *et al.*, 2004, 2005), lateral body shape is also known to exhibit phenotypic plasticity in response to changes in

diet and foraging orientation (Ruehl & DeWitt, 2005). Because no study had previously assessed lateral body shape of adult laboratory-reared individuals, whether divergence in lateral shape between predator regimes reflected genetically based differentiation remained largely an open question prior to this study. I found that laboratory-reared adult *G. affinis* derived from populations coexisting with predatory fish exhibited smaller anterior body/head regions, larger caudal regions and a more posterior placement of the eye compared with fish derived from populations lacking piscivorous fish. These differences not only match those observed in wild-caught individuals, but variation in anterior body/head size and caudal peduncle size also match *a priori* predictions. That is, combining biomechanical knowledge of the functional links between body form and swimming performance with ecological knowledge of how swimming performance should mediate fitness in alternative predator regimes – i.e. selection should favour steady swimming in low-predation environments, but favour unsteady swimming in high-predation environments – yields the prediction of smaller anterior body/head regions and larger caudal regions in high-predation environments, as observed (see Langerhans & Reznick, in press). This correspondence between predictions and observations strongly argues for DNS between predator regimes as the primary mechanism of divergence, and is inconsistent with other explanations such as genetic drift (e.g. Endler, 1986; Wainwright, 1988, 1996; Losos, 1990; Williams, 1992; Walker, 1997; Domenici, 2003). Further, recent work is uncovering empirical data from several other systems that also confirm these predictions, suggesting such predator-driven divergence might represent an ecomorphological paradigm, where similar forms of DNS between predator regimes repeatedly drive similar cases of morphological divergence within distantly related lineages (e.g. Langerhans *et al.*, 2004; Gomes & Monteiro, 2008; reviewed in Langerhans & Reznick, in press).

Although overall trends match predictions, it is unclear exactly what selection pressures might be responsible for the observed differences in positioning of the eye. Eye placement might be important for prey acquisition or predator detection, but no known selective differences between predator regimes would favour such differences in eye location, and thus deserve further study. Additionally, without testing the functional consequences of morphological differences, it is possible that the predicted body shapes have evolved for alternative reasoning (i.e. some mechanism other than DNS for steady and unsteady swimming between predator regimes). That is, one could get the ‘right’ result for the ‘wrong’ reasons. For instance, differences in anterior body/head size might be associated with shifts in diet (e.g. consumption or digestion of different prey types; Bouton *et al.*, 2002) or foraging mode (e.g. suction, ram, scraping/picking; Liem, 1980). Dietary shifts have not yet been explored in

this system, and thus their potential role is unknown. It is for reasons such as these that the examination of functional consequences of morphological variation (e.g. swimming performance) is so important.

Steady-swimming performance

Because *Gambusia* fishes use steady swimming during most competitive activities (see Introduction), steady-swimming performance should greatly influence competitive interactions (which generally strengthen under the higher densities common to environments with low extrinsic mortality rates). Moreover, in low-predation environments, *Gambusia* often exhibit high densities and utilize open-water habitat (e.g. Winkelman & Aho, 1993; Langerhans, 2006), putting a premium on the ability to continually cruise through their environment in search of limited resources. Thus, fish with relatively high steady-swimming performance are predicted to exhibit high fitness in low-predation environments (i.e. obtain and consume food more quickly, acquire mates more effectively, contain greater energy supplies for reproduction) (e.g. Vogel, 1994; Plaut, 2001; Domenici, 2003; Blake, 2004).

I found that morphological differences between laboratory-reared *G. affinis* derived from different predatory environments resulted in different manners of thrust production during steady swimming, consequently leading to differences in endurance. Compared with fish derived from low-predation environments, fish from high-predation environments exhibited more flexible bodies (lower propulsive wavelength) as predicted, yielding slower wave speeds at a given driving frequency. To compensate for their more flexible bodies and higher drag, high-predation fish increased driving amplitude (higher rostral amplitude, resulting in higher tail-beat amplitude) but not driving frequency (similar tail-beat frequency). This shift in swimming kinematics resulted in less efficient thrust generation (higher power consumption) and lower endurance (lower fatigue time). Thus, consistent with *a priori* predictions based on DNS between predator regimes, fish derived from high-predation environments – which are known to exhibit higher unsteady-swimming performance – suffer reduced steady-swimming abilities relative to fish from low-predation environments.

Although I did not directly measure flexural stiffness of the body, propulsive wavelength should provide a reasonable surrogate for body stiffness as it is body stiffness that is believed to largely control the shape of the undulatory wave (Long *et al.*, 1994; McHenry *et al.*, 1995; Long & Nipper, 1996). Consistent with the conclusion of stiffer bodies in *G. affinis* individuals derived from low-predation environments, low-predation fish also exhibit lower turning angles during stage I of a C-start escape response compared with high-predation fish, suggesting their bodies are indeed less flexible

(R.B. Langerhans, unpublished). Owing to their stiffer bodies, low-predation fish apparently employed a more energetically efficient method of thrust production, which exploits stored energy in the spring-like scenario present in stiff bodies (Long & Nipper, 1996; Pabst, 1996; Dickinson *et al.*, 2000). This method of thrust generation is presumably less metabolically costly than the production of similar levels of thrust via increases in driving frequency or amplitude; however, this hypothesis has yet to be tested. Although it is clear that low-predation fish have stiffer bodies during steady swimming relative to high-predation fish, exactly how this difference has arisen is unclear. That is, differences in stiffness could derive from the increased ability of low-predation fish to actively stiffen their bodies during swimming using negative work, from increased passive flexural stiffness in low-predation fish (e.g. involving differences in muscles, skin) or a combination of both mechanisms (Blight, 1976; Van Leeuwen *et al.*, 1990; Altringham *et al.*, 1993; Long *et al.*, 1996).

It is notable that driving amplitude and not driving frequency differed between fish inhabiting different predator regimes. Tail-beat frequency is probably the most commonly studied kinematic variable in steady swimming, and is often thought to reflect variation in oxygen consumption among closely related fish (which are typically assumed to exhibit similarities in other kinematic variables) (e.g. Herskin & Steffensen, 1998; Lowe, 2001; Steinhausen *et al.*, 2005; Ohlberger *et al.*, 2007). However, as this study highlights, fish can modify other kinematic variables to modulate thrust generation, and tail-beat frequency alone may be insufficient in some cases for characterization of energy costs such as active metabolic rates. Here, *G. affinis* derived from high-predation environments exhibited similar tail-beat frequencies as fish from low-predation environments, but yet still appear to have required greater oxygen consumption while swimming at a common speed (i.e. higher relative power, lower fatigue time; see below) owing to their increased driving amplitude. Although I have assumed that rostral amplitude provides a useful surrogate for driving amplitude, it could also be interpreted as largely a recoil response to lateral motion of the body (Lighthill, 1970; Webb, 1988). More likely, it probably reflects both sources of variation as they are inextricably linked (i.e. the higher the driving amplitude, the larger the recoil response to it). Thus, the larger amplitude undulatory waves (reflected by increased rostral and tail-beat amplitude) observed in high-predation environments surely reflect greater driving amplitude compared to low-predation fish.

Results suggest that low-predation fish have lower metabolic costs during steady swimming than fish derived from high-predation localities. Although direct measurement of metabolic power is needed to confirm this conclusion, endurance estimates should be positively correlated with metabolic power. This is because fatigue

time presumably largely reflects maximum aerobic capacity, and therefore the upper limit of metabolic scope (Beamish, 1978). I found that fish with higher fatigue time tended to exhibit greater three-dimensional streamlining, larger heads, smaller caudal peduncles, higher wave speeds and lower amplitude undulations. Thus, it is reasonable to conclude that fish derived from low-predation environments suffered lower metabolic costs of transport than high-predation fish swimming at the same speed.

Trade-off between steady and unsteady locomotion

A functional trade-off is manifested when a given morphological or physiological trait exhibits oppositely-signed relationships with two performance variables (e.g. see Walker, 2007; Langerhans, 2008). A general prediction in fish hydrodynamics is that a functional trade-off exists between steady and unsteady locomotion for fishes employing a coupled locomotor system. I focused here on the coupling of morphological features: the same body parts being used for propulsion in both steady-swimming and unsteady-swimming activities. Theory indicates that steady swimming is optimized with a stiff, streamlined body having a large anterior body/head region and a small caudal region, whereas unsteady swimming is optimized with the opposite trait values (e.g. Webb, 1982, 1984; Videler, 1993; Walker, 1997; Blake, 2004; Langerhans & Reznick, in press). Empirical work with *G. affinis* is consistent with these theoretical predictions: populations exhibiting stiffer, more streamlined bodies, with larger anterior body/head regions and smaller caudal regions also display higher steady-swimming performance but lower unsteady-swimming performance (Fig. 7). Importantly, these relationships between morphology and swimming performance also appear to hold both between and *within* populations, suggesting a causal relationship. That is, fish with larger caudal regions and smaller anterior body/head regions produce higher burst-swimming speeds – a trend consistently observed within multiple populations (Langerhans *et al.*, 2004) – but expend greater hydromechanical power during steady swimming, resulting in reduced endurance – again, a trend consistently observed within multiple populations (this study). Although this indicates the existence of a functional trade-off, it does not directly demonstrate a performance trade-off across individuals, as the two types of swimming modes were not measured on the same fish (e.g. correlated traits might mask the trade-off across individuals, see below). Thus, the observed functional trade-off predicts the occurrence of an individual-level performance trade-off, but does not demonstrate it – although prior evidence does exist for this trade-off in *G. affinis* (see Langerhans, 2006). Based on the observed form–function relationships, combined with the hypothesized DNS on steady and unsteady locomotion between predator regimes, we have *a priori*

predictions for divergent phenotypic evolution, including stiffer, more streamlined bodies, larger anterior body/head regions and smaller caudal regions in environments with lower predation intensity. This study revealed that morphological divergence between predatory environments in *G. affinis* matches all these predictions.

Whether the trade-off between steady and unsteady swimming is common to most fish is currently unclear. Based on the functional morphology of fish locomotion, the trade-off should exist whenever propulsor mechanisms are highly coupled. Although the great majority of fish exhibit some degree of locomotor coupling (it is virtually impossible to completely decouple all propulsors), some species have evolved locomotor systems with varying degrees of independence among swimming modes. Most commonly, fish employ median-and-paired fin propulsion for certain swimming activities, such as low-speed cruising or burst-and-coast swimming, and body-and-caudal fin propulsion for most other activities (e.g. Webb, 1998; Sfakiotakis *et al.*, 1999; Drucker & Lauder, 2000; Hove *et al.*, 2001; Blake, 2004). Further, numerous features of fish design influence locomotor performance (e.g. body form, fin size/shape, muscle size/type, gill size; see Webb, 1998; Domenici, 2003; Lauder, 2005; Langerhans & Reznick, in press), allowing for the possibility of many-to-one mapping (Wainwright *et al.*, 2005; Wainwright, 2007). That is, multiple combinations of traits might be capable of producing equivalent levels of locomotor performance, effectively disguising

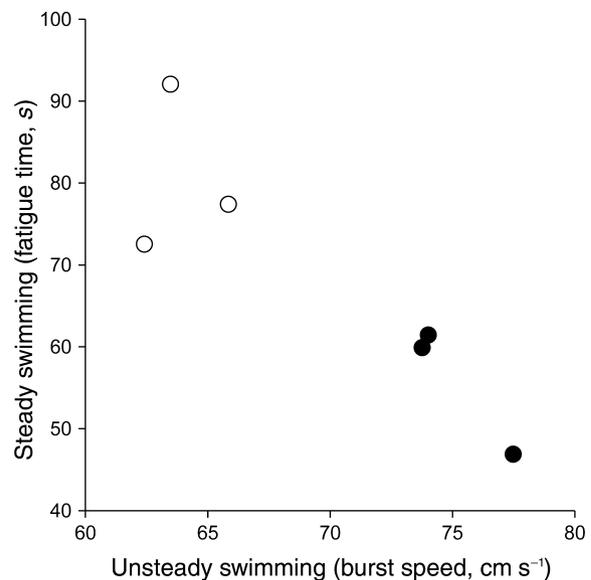


Fig. 7 Trade-off between steady- and unsteady-swimming performance across six *Gambusia affinis* populations. Open symbols represent low-predation populations and filled symbols represent high-predation populations. Values are population means given at a common body length of 28.0 mm. The two performance variables are significantly associated ($r = -0.89$, one-tailed $P = 0.009$). Data for burst-swimming speed are from Langerhans *et al.* (2004).

the trade-off at the whole-organism level despite the fact that strong trade-offs might exist at all lower levels of the phenotypes themselves. This means that the performance trade-off should weaken as traits become more independent of one another (e.g. lower genetic correlation among lower-level traits). Further, inter-individual variation in general quality or motivation can mask the existence of a trade-off (e.g. Van Noordwijk & de Jong, 1986; Losos *et al.*, 2002; Van Damme *et al.*, 2002). Together, integrated forms of propulsion, many-to-one mapping and confounding factors related to inter-individual variation serve to obfuscate theoretical predictions regarding a trade-off between steady and unsteady locomotion, making the strength of the trade-off largely an empirical question in need of proper experimental design. A number of studies to date have examined general endurance-sprint trade-offs in diverse taxa, and although a number of studies have found evidence of a trade-off (e.g. Huey *et al.*, 1984; Arnold & Bennett, 1988; Garland *et al.*, 1988; Dohm *et al.*, 1996; Reidy *et al.*, 2000; Vanhooydonck *et al.*, 2001; Van Damme *et al.*, 2002; Wilson *et al.*, 2002, 2004; Ojanguren & Brana, 2003; Yang *et al.*, 2003; Santiago *et al.*, 2008), many have not (e.g. Bennett, 1980; Ford & Shuttlesworth, 1986; Garland & Else, 1987; Garland, 1988; Bennett *et al.*, 1989; Tsuji *et al.*, 1989; Huey *et al.*, 1990; Jayne & Bennett, 1990; Secor *et al.*, 1992; Sorci *et al.*, 1995; Pinch & Claussen, 2003). This suggests that such a trade-off indeed depends on numerous underlying factors, leaving its strength unpredictable without empirical data.

Assuming this trade-off does indeed apply to many fishes, an interesting question is whether DNS between steady and unsteady swimming might represent a generally important diversifying mechanism in fishes. Langerhans & Reznick (in press) recently reviewed the existing evidence for this hypothesis and posited that this particular form of DNS might be of paramount importance in fish diversification, pointing to multiple environmental gradients, including predation, that might commonly generate such DNS. Thus, it seems that the role of DNS involving steady and unsteady locomotion in driving evolutionary divergence and speciation in fishes may be of great significance and demands focused attention.

Rapid evolution, invasiveness and speciation

Although exact dates are unknown, it is likely that populations examined here were colonized and isolated from one another within the last 150 years when human-induced changes in the area led to an overall reduction in surface waters and an increase in isolated, semi-permanent aquatic environments (Schmidly, 2002). This suggests that evolutionary changes documented here occurred quite rapidly (and likely in the face of at least some level of gene flow given the spatial proximity of some divergent populations, e.g. 0.80 km). Using Haldanes (trait

change in standard deviation units per generation) as an estimate of evolutionary rate, some traits have diverged as much as twice that expected from other studies of microevolution (predicted change of ~ 0.66 standard deviations over an estimated 200–300 generations according to a large data set described in Kinnison & Hendry, 2001), and are similar to that previously documented for life history traits in introduced populations of *G. affinis* (Stockwell *et al.*, 2003). While this study examined populations of *G. affinis* within its native range, *G. affinis* and another *Gambusia* species, *G. holbrooki*, have been introduced worldwide, and are highly invasive in many areas (e.g. Krumholz, 1948; Courtenay & Meffe, 1989; Gamradt & Kats, 1996; Lever, 1996; Webb & Joss, 1997; Goodsell & Kats, 1999; ISSG, 2000; Mills *et al.*, 2004; Laha & Mattingly, 2007). Given the results of this study, combined with the previous demonstration of rapid evolution in introduced *G. affinis* populations (Stockwell & Weeks, 1999), the ability for such rapid, adaptive evolutionary responses may play an important role in the widespread native range of *G. affinis* and its invasiveness worldwide by facilitating successful colonization of novel environments (e.g. reviewed in Mooney & Cleland, 2001; Reznick & Ghalambor, 2001; Sakai *et al.*, 2001; Lee, 2002; Stockwell *et al.*, 2003; Cox, 2004; Huey *et al.*, 2005; Wares *et al.*, 2005).

Cases of adaptive differentiation, as observed here, not only provide the basis for much of the phenotypic diversity we see today but also centre prominently in the process of ecological speciation (Schluter, 2001; Rundle & Nosil, 2005; Podos & Hendry, 2006; Schluter, 2009). Ecological speciation describes the evolution of barriers to gene flow resulting from ecologically based divergent selection. Recent work has implicated DNS between predator regimes as a possible driver of ecological speciation in *Gambusia* fishes (Langerhans *et al.*, 2007), and work in other systems has demonstrated that ecological speciation can commence within only dozens of generations (Hendry *et al.*, 2007). Moreover, ongoing work across the *Gambusia* genus is revealing strong evidence for repeated patterns of morphological evolution within similar predator regimes (R.B. Langerhans, unpublished). This suggests that the microevolutionary changes documented here might be mirrored at the interspecific level. Irrespective of whether results of this study are more relevant as the catalyst of invasiveness, the driving force of speciation, or transient evolutionary blips across a geographical mosaic of communities, the process of DNS driving evolutionary change is the same in all cases, and the ecological and evolutionary importance of this process is undisputed.

Acknowledgments

I thank A. Hendry, J. Losos and an anonymous reviewer for comments on a previous draft of the manuscript, and

G. Lauder and K. Liem for beneficial discussions regarding this work. This research was funded by a U.S. Environmental Protection Agency Science to Achieve Results (STAR) fellowship, a National Science Foundation grant (DEB-0722480), and a Society of Integrative and Comparative Biology Grant in Aid of Research.

References

- Abrams, P.A. 2001. The effect of density-independent mortality on the coexistence of exploitative competitors for renewing resources. *Am. Nat.* **158**: 459–470.
- Altringham, J.D., Wardle, C.S. & Smith, C.I. 1993. Myotomal muscle function at different locations in the body of a swimming fish. *J. Exp. Biol.* **182**: 191–206.
- Arbuckle, J.L. 2003. *Amos 5.0 Update to the Amos User's Guide*. Smallwaters Corporation, Chicago, IL.
- Arbuckle, J.L. & Wothke, W. 1999. *Amos 4.0 User's Guide*. Smallwaters Corporation, Chicago, IL.
- Arnold, S.J. & Bennett, A.F. 1988. Behavioral variation in natural populations. V. Morphological correlates of locomotion in the garter snake (*Thamnophis radix*). *Biol. J. Linn. Soc.* **34**: 175–190.
- Beamish, F.W.H. 1978. Swimming capacity. In: *Fish Physiology*, Vol. 7 (W.S. Hoar & J.D. Randall, eds), pp. 101–187. Academic Press Inc., New York.
- Bennett, A.F. 1980. The thermal dependence of lizard behavior. *Anim. Behav.* **28**: 752–762.
- Bennett, A.F., Garland, T. & Else, P.L. 1989. Individual correlation of morphology, muscle mechanics, and locomotion in a salamander. *Am. J. Physiol.* **256**: R1200–R1208.
- Blake, R.W. 1983. *Fish Locomotion*. Cambridge University Press, Cambridge.
- Blake, R.W. 2004. Fish functional design and swimming performance. *J. Fish Biol.* **65**: 1193–1222.
- Blake, R.W., Law, T.C., Chan, K.H.S. & Li, J.F.Z. 2005. Comparison of the prolonged swimming performances of closely related, morphologically distinct three-spined sticklebacks *Gasterosteus* spp. *J. Fish Biol.* **67**: 834–848.
- Blight, A.R. 1976. Undulatory swimming with and without waves of contraction. *Nature* **264**: 352–354.
- Bollen, K.A. & Stine, R.A. 1992. Bootstrapping goodness-of-fit measures in structural equation models. *Sociol. Methods Res.* **21**: 205–229.
- Bookstein, F.L. 1991. *Morphometric Tools for Landmark Data*. Cambridge University Press, New York.
- Bookstein, F.L. 1996. Combining the tools of geometric morphometrics. In: *Advances in Morphometrics* (L.F. Marcus, M. Corti, A. Loy, G.J.P. Naylor & D.E. Slice, eds), pp. 131–151. Plenum Press, New York.
- Bouton, N., De Visser, J. & Barel, C.D.N. 2002. Correlating head shape with ecological variables in rock-dwelling haplochromines (Teleostei: Cichlidae) from Lake Victoria. *Biol. J. Linn. Soc.* **76**: 39–48.
- Brainerd, E.L. & Patek, S.N. 1998. Vertebral column morphology, C-start curvature, and the evolution of mechanical defenses in tetraodontiform fishes. *Copeia* **1998**: 971–984.
- Chesson, P. & Huntly, N. 1997. The roles of harsh and fluctuating conditions in the dynamics of ecological communities. *Am. Nat.* **150**: 519–553.
- Cohen, J.E. 1988. *Statistical Power Analysis for the Behavioral Sciences*, 2nd edn. Lawrence Earlbaum Associates, Hillsdale, NJ.
- Courtenay, W.R. & Meffe, G.K. 1989. Small fishes in strange places: a review of introduced poeciliids. In: *Ecology and Evolution of Livebearing Fishes (Poeciliidae)* (G.K. Meffe & F.F. Snelson, eds), pp. 319–331. Prentice Hall, Englewood Cliffs, NJ.
- Cox, G.W. 2004. *Alien Species and Evolution*. Island Press, Washington, DC.
- Cumming, G. & Finch, S. 2001. A primer on the understanding, use, and calculation of confidence intervals that are based on central and noncentral distributions. *Educ. Psychol. Meas.* **61**: 532–574.
- Dickinson, M.H., Farley, C.T., Full, R.J., Koehl, M.A.R., Kram, R. & Lehman, S. 2000. How animals move: an integrative view. *Science* **288**: 100–106.
- Dohm, M.R., Hayes, J.P. & Garland, T. 1996. Quantitative genetics of sprint running speed and swimming endurance in laboratory house mice (*Mus domesticus*). *Evolution* **50**: 1688–1701.
- Domenici, P. 2003. Habitat, body design and the swimming performance of fish. In: *Vertebrate Biomechanics and Evolution* (V.L. Bels, J.-P. Gasc & A. Casinos, eds), pp. 137–160. BIOS Scientific Publishers Ltd, Oxford.
- Domenici, P. 2009. Escape responses in fish: kinematics, performance, and behavior. In: *Fish Locomotion: An Etho-Ecological Perspective* (P. Domenici & B.G. Kapoor, eds), Science Publishers, Enfield, CT, in press.
- Domenici, P. & Blake, R.W. 1997. The kinematics and performance of fish fast-start swimming. *J. Exp. Biol.* **200**: 1165–1178.
- Drucker, E.G. & Lauder, G.V. 2000. A hydrodynamic analysis of fish swimming speed: wake structure and locomotor force in slow and fast labriform swimmers. *J. Exp. Biol.* **203**: 2379–2393.
- Dryden, I.L. & Mardia, K.V. 1998. *Statistical Shape Analysis*. John Wiley & Sons, New York.
- Eaton, R.C., Bombardieri, R.A. & Meyer, D.L. 1977. The Mauthner-initiated startle response in teleost fish. *J. Exp. Biol.* **66**: 65–81.
- Endler, J.A. 1986. *Natural Selection in the Wild*. Princeton University Press, Princeton, NJ.
- Endler, J.A. 1995. Multiple-trait coevolution and environmental gradients in guppies. *Trends Ecol. Evol.* **10**: 22–29.
- Fidler, F. & Thompson, B. 2001. Computing correct confidence intervals for ANOVA fixed- and random-effects effect sizes. *Educ. Psychol. Meas.* **61**: 575–604.
- Fisher, R. & Hogan, J.D. 2007. Morphological predictors of swimming speed: a case study of pre-settlement juvenile coral reef fishes. *J. Exp. Biol.* **210**: 2436–2443.
- Ford, N.B. & Shuttlesworth, G.A. 1986. Effects of variation in food intake on locomotory performance of juvenile garter snakes. *Copeia* **1986**: 999–1001.
- Frontier, S. 1976. Étude de la décroissance des valeurs propres dans une analyse en composantes principales: comparaison avec le modle du bâton brisé. *J. Exp. Mar. Biol. Ecol.* **25**: 67–75.
- Gamradt, S.C. & Kats, L.B. 1996. Effect of introduced crayfish and mosquitofish on California newts. *Conserv. Biol.* **10**: 1155–1162.

- Garland, T. 1988. Genetic basis of activity metabolism. I. Inheritance of speed, stamina, and antipredator displays in the garter snake *Thamnophis sirtalis*. *Evolution* **42**: 335–350.
- Garland, T. & Else, P.L. 1987. Seasonal, sexual, and individual variation in endurance and activity metabolism in lizards. *Am. J. Physiol.* **252**: R439–R449.
- Garland, T. & Losos, J.B. 1994. Ecological morphology of locomotor performance in squamate reptiles. In: *Ecological Morphology: Integrative Organismal Biology* (P.C. Wainwright & S.M. Reilly, eds), pp. 240–302. University of Chicago Press, Chicago, IL.
- Garland, T., Geiser, F. & Baudinette, R.V. 1988. Comparative locomotor performance of marsupial and placental mammals. *J. Zool.* **215**: 505–522.
- Ghalambor, C.K., Reznick, D.N. & Walker, J.A. 2004. Constraints on adaptive evolution: the functional trade-off between reproduction and fast-start swimming performance in the Trinidadian guppy (*Poecilia reticulata*). *Am. Nat.* **164**: 38–50.
- Gomes, J.L. & Monteiro, L.R. 2008. Morphological divergence patterns among populations of *Poecilia vivipara* (Teleostei Poeciliidae): test of an ecomorphological paradigm. *Biol. J. Linn. Soc.* **93**: 799–812.
- Goodsell, J.A. & Kats, L.B. 1999. Effect of introduced mosquitofish on pacific treefrogs and the role of alternative prey. *Conserv. Biol.* **13**: 921–924.
- Gurevitch, J., Morrison, J.A. & Hedges, L.V. 2000. The interaction between competition and predation: a meta-analysis of field experiments. *Am. Nat.* **155**: 435–453.
- Hale, M.E., Long, J.H., McHenry, M.J. & Westneat, M.W. 2002. Evolution of behavior and neural control of the fast-start escape response. *Evolution* **56**: 993–1007.
- Hassell, M.P. 1975. Density-dependence in single-species populations. *J. Anim. Ecol.* **44**: 283–295.
- Hendry, A.P., Nosil, P. & Rieseberg, L.H. 2007. The speed of ecological speciation. *Funct. Ecol.* **21**: 455–464.
- Herskin, J. & Steffensen, J.F. 1998. Energy savings in sea bass swimming in a school: measurements of tail beat frequency and oxygen consumption at different swimming speeds. *J. Fish Biol.* **53**: 366–376.
- Hoerner, S.F. 1965. *Fluid-Dynamic Drag*. Published by the author, Midland Park.
- Holt, R.D. 1985. Density-independent mortality, non-linear competitive interactions, and species coexistence. *J. Theor. Biol.* **116**: 479–493.
- Horwood, J.W. & Cushing, D.H. 1977. Spatial distributions and ecology of pelagic fish. In: *Spatial patterns in plankton communities* (J.H. Steele, ed.), pp. 355–383. Plenum Press, New York.
- Hove, J.R., O'Bryan, L.M., Gordon, M.S., Webb, P.W. & Weihs, D. 2001. Boxfishes (Teleostei: Ostraciidae) as a model system for fishes swimming with many fins: kinematics. *J. Exp. Biol.* **204**: 1459–1471.
- Huey, R.B., Bennett, A.F., Johnalder, H. & Nagy, K.A. 1984. Locomotor capacity and foraging behavior of Kalahari lacertid lizards. *Anim. Behav.* **32**: 41–50.
- Huey, R.B., Dunham, A.E., Overall, K.L. & Newman, R.A. 1990. Variation in locomotor performance in demographically known populations of the lizard *Sceloporus merriami*. *Physiol. Zool.* **63**: 845–872.
- Huey, R.B., Gilchrist, G.W. & Hendry, A.P. 2005. Using invasive species to study evolution: case studies with *Drosophila* and salmon. In: *Species Invasions. Insights into Ecology, Evolution, and Biogeography* (D.F. Sax, J.J. Stachowicz & S.D. Gaines, eds), pp. 139–164. Sinauer Associates, Inc., Sunderland, MA.
- ISSG. 2000. *100 of the World's Worst Invasive Alien Species: A Selection from the Global Invasive Species Database*, Invasive Species Specialist Group, University of Auckland, Auckland.
- Jackson, D.A. 1993. Stopping rules in principal components analysis: a comparison of heuristic and statistical approaches. *Ecology* **74**: 2204–2214.
- Jayne, B.C. & Bennett, A.F. 1990. Selection on locomotor performance capacity in a natural population of garter snakes. *Evolution* **44**: 1204–1229.
- Jennions, M.D. & Telford, S.R. 2002. Life-history phenotypes in populations of *Brachyrhaphis episcopi* (Poeciliidae) with different predator communities. *Oecologia* **132**: 44–50.
- Johnson, J.B. & Belk, M.C. 2001. Predation environment predicts divergent life-history phenotypes among populations of the livebearing fish *Brachyrhaphis rhabdophora*. *Oecologia* **126**: 142–149.
- Johnson, J.B., Burt, D.B. & DeWitt, T.J. 2008. Form, function, and fitness: pathways to survival. *Evolution* **62**: 1243–1251.
- Katzir, G. & Camhi, J.M. 1993. Escape response of black mollies (*Poecilia shenops*) to predatory dives of a pied kingfisher (*Ceryle rudis*). *Copeia* **1993**: 549–553.
- Kinnison, M.T. & Hendry, A.P. 2001. The pace of modern life II: from rates of contemporary microevolution to pattern and process. *Genetica* **112**: 145–164.
- Kline, R.B. 2005. *Principles and Practice of Structural Equation Modeling*, 2nd edn. The Guilford Press, New York.
- Klingenberg, C.P. & Monteiro, L.R. 2005. Distances and directions in multidimensional shape spaces: implications for morphometric applications. *Syst. Biol.* **54**: 678–688.
- Klingenberg, C.P., Barluenga, M. & Meyer, A. 2003. Body shape variation in cichlid fishes of the *Amphilophus citrinellus* species complex. *Biol. J. Linn. Soc.* **80**: 397–408.
- Krumholz, L.A. 1948. Reproduction in the western mosquitofish *Gambusia affinis affinis* (Baird and Girard) and its use in mosquito control. *Ecol. Monogr.* **18**: 1–43.
- Laha, M. & Mattingly, H.T. 2007. Ex situ evaluation of impacts of invasive mosquitofish on the imperiled Barrens topminnow. *Environ. Biol. Fishes* **78**: 1–11.
- Langerhans, R.B. 2006. Evolutionary consequences of predation: avoidance, escape, reproduction, and diversification. In: *Predation in Organisms: A Distinct Phenomenon* (A.M.T. Elewa, ed.), pp. 177–220. Springer-Verlag, Heidelberg, Germany.
- Langerhans, R.B. 2008. Predictability of phenotypic differentiation across flow regimes in fishes. *Integr. Comp. Biol.* **48**: 750–768.
- Langerhans, R.B. & DeWitt, T.J. 2004. Shared and unique features of evolutionary diversification. *Am. Nat.* **164**: 335–349.
- Langerhans, R.B. & Reznick, D.N. 2009. Ecology and evolution of swimming performance in fishes: predicting evolution with biomechanics. In: *Fish Locomotion: An Etho-ecological Perspective* (P. Domenici & B.G. Kapoor, eds). Science Publishers, Enfield, CT, in press.
- Langerhans, R.B., Layman, C.A., Shokrollahi, A.M. & DeWitt, T.J. 2004. Predator-driven phenotypic diversification in *Gambusia affinis*. *Evolution* **58**: 2305–2318.
- Langerhans, R.B., Layman, C.A. & DeWitt, T.J. 2005. Male genital size reflects a tradeoff between attracting mates and

- avoiding predators in two live-bearing fish species. *Proc. Natl Acad. Sci. USA* **102**: 7618–7623.
- Langerhans, R.B., Gifford, M.E. & Joseph, E.O. 2007. Ecological speciation in *Gambusia* fishes. *Evolution* **61**: 2056–2074.
- Lauder, G.V. 2005. Locomotion. In: *The Physiology of Fishes* (D.H. Evans & J.B. Claiborne, eds), pp. 3–46. CRC Press, Boca Raton, FL.
- Lee, C.E. 2002. Evolutionary genetics of invasive species. *Trends Ecol. Evol.* **17**: 386–391.
- Lever, C. 1996. *Naturalized Fishes of the World*. Academic, New York.
- Liem, K.F. 1980. Acquisition of energy by teleosts: adaptive mechanisms and evolutionary patterns. In: *Environmental Physiology of Fishes* (M.A. Ali, ed.), pp. 299–334. Plenum Press, New York.
- Lighthill, M.J. 1969. Hydromechanics of aquatic animal propulsion: a survey. *A. Rev. Fluid Mech.* **1**: 413–446.
- Lighthill, M.J. 1970. Aquatic animal propulsion of high hydro-mechanical efficiency. *J. Fluid Mech.* **44**: 265–301.
- Lighthill, M.J. 1975. *Mathematical Biofluid Dynamics*. Society for Applied and Industrial Mathematics, Philadelphia.
- Long, J.H. & Nipper, K.S. 1996. The importance of body stiffness in undulatory propulsion. *Am. Zool.* **36**: 678–694.
- Long, J.H., McHenry, M.J. & Boetticher, N.C. 1994. Undulatory swimming: how traveling waves are produced and modulated in sunfish (*Lepomis gibbosus*). *J. Exp. Biol.* **192**: 129–145.
- Long, J.H., Hale, M.E., McHenry, M.J. & Westneat, M.W. 1996. Functions of fish skin: flexural stiffness and steady swimming of longnose gar *Lepisosteus osseus*. *J. Exp. Biol.* **199**: 2139–2151.
- Losos, J.B. 1990. The evolution of form and function: morphology and locomotor performance in West Indian *Anolis* lizards. *Evolution* **44**: 1189–1203.
- Losos, J.B., Creer, D.A. & Schulte, J.A. 2002. Cautionary comments on the measurement of maximum locomotor capabilities. *J. Zool.* **258**: 57–61.
- Lowe, C.G. 2001. Metabolic rates of juvenile scalloped hammerhead sharks (*Sphyrna lewini*). *Mar. Biol.* **139**: 447–453.
- Marcus, L.F., Corti, M., Loy, A., Naylor, G.J.P. & Slice, D.E. 1996. *Advances in Morphometrics*. Plenum Press, New York.
- McHenry, M.J. & Lauder, G.V. 2006. Ontogeny of form and function: locomotor morphology and drag in zebrafish (*Danio rerio*). *J. Morphol.* **267**: 1099–1109.
- McHenry, M.J., Pell, C.A. & Long, J.H. 1995. Mechanical control of swimming speed: stiffness and axial wave form in undulating fish models. *J. Exp. Biol.* **198**: 2293–2305.
- Meffe, G.K. & Snelson, F.F. 1989. An ecological overview of poeciliid fishes. In: *Ecology and Evolution of Livebearing Fishes (Poeciliidae)* (G.K. Meffe & F.F. Snelson, eds), pp. 13–31. Prentice Hall, Englewood Cliffs, NJ.
- Mills, M.D., Rader, R.B. & Belk, M.C. 2004. Complex interactions between native and invasive fish: the simultaneous effects of multiple negative interactions. *Oecologia* **141**: 713–721.
- von Mises, R. 1945. *Theory of Flight*. Dover Books, New York.
- Mitchell, R.J. 1992. Testing evolutionary and ecological hypotheses using path analysis and structural equation modeling. *Funct. Ecol.* **6**: 123–129.
- Mitchell, R.J. 2001. Path analysis: pollination. In: *Design and Analysis of Ecological Experiments* (S.M. Scheiner & J. Gurevitch, eds), pp. 217–234. Oxford University Press, Oxford.
- Mooney, H.A. & Cleland, E.E. 2001. The evolutionary impact of invasive species. *Proc. Natl Acad. Sci. USA* **98**: 5446–5451.
- Niimi, A.J. & Beamish, F.W.H. 1974. Bioenergetics and growth of largemouth bass *Micropterus salmoides*. *Can. J. Zool.* **52**: 447–456.
- Nikora, V.I., Aberle, J., Biggs, B.J.F., Jowett, I.G. & Sykes, J.R.E. 2003. Effects of fish size, time-to-fatigue and turbulence on swimming performance: a case study of *Galaxias maculatus*. *J. Fish Biol.* **63**: 1365–1382.
- O’Steen, S., Cullum, A.J. & Bennett, A.F. 2002. Rapid evolution of escape ability in Trinidadian guppies (*Poecilia reticulata*). *Evolution* **56**: 776–784.
- Ohlberger, J., Staaks, G. & Holker, F. 2007. Estimating the active metabolic rate (AMR) in fish based on tail beat frequency (TBF) and body mass. *J. Exp. Zool. Part A Ecol. Genet. Physiol.* **307A**: 296–300.
- Ojanguren, A.F. & Brana, F. 2003. Effects of size and morphology on swimming performance in juvenile brown trout (*Salmo trutta* L.). *Ecol. Freshw. Fish.* **12**: 241–246.
- Orr, M.R. & Smith, T.B. 1998. Ecology and speciation. *Trends Ecol. Evol.* **13**: 502–506.
- Pabst, D.A. 1996. Springs in swimming animals. *Am. Zool.* **36**: 723–735.
- Pedley, T.J. & Hill, S.J. 1999. Large-amplitude undulatory fish swimming: fluid mechanics coupled to internal mechanics. *J. Exp. Biol.* **202**: 3431–3438.
- Pinch, F.C. & Claussen, D.L. 2003. Effects of temperature and slope on the sprint speed and stamina of the Eastern Fence Lizard, *Sceloporus undulatus*. *J. Herpetol.* **37**: 671–679.
- Plaut, I. 2001. Critical swimming speed: its ecological relevance. *Comp. Biochem. Physiol. A Mol. Integr. Physiol.* **131**: 41–50.
- Podos, J. & Hendry, A.P. 2006. The biomechanics of ecological speciation. In: *Ecology and Biomechanics: A Mechanical Approach to the Ecology of Animals and Plants* (A. Herrel, T. Speck & N. Rowe, eds), pp. 301–321. Taylor and Francis, New York.
- Reidy, S., Kerr, S. & Nelson, J. 2000. Aerobic and anaerobic swimming performance of individual Atlantic cod. *J. Exp. Biol.* **203**: 347–357.
- Reznick, D.N. & Endler, J.A. 1982. The impact of predation on life history evolution in Trinidadian guppies (*Poecilia reticulata*). *Evolution* **36**: 160–177.
- Reznick, D.N. & Ghalambor, C.K. 2001. The population ecology of contemporary adaptations: what empirical studies reveal about the conditions that promote adaptive evolution. *Genetica* **112**: 183–198.
- Reznick, D.N., Shaw, F.H., Rodd, F.H. & Shaw, R.G. 1997. Evaluation of the rate of evolution in natural populations of guppies (*Poecilia reticulata*). *Science* **275**: 1934–1937.
- Reznick, D.N., Butler, M.J. & Rodd, H. 2001. Life-history evolution in guppies. VII. The comparative ecology of high- and low-predation environments. *Am. Nat.* **157**: 126–140.
- Rice, W.R. & Hostert, E.E. 1993. Laboratory experiments on speciation: what have we learned in 40 years? *Evolution* **47**: 1637–1653.
- Robinson, B.W. & Wilson, D.S. 1994. Character release and displacement in fishes: a neglected literature. *Am. Nat.* **144**: 596–627.
- Rohlf, F.J. 2005. *TpsRegr*. Department of Ecology and Evolution, State University, Stony Brook, New York.
- Rohlf, F.J. 2006. *TpsDig*. Department of Ecology and Evolution, State University, Stony Brook, New York.
- Rohlf, F.J. & Marcus, L.F. 1993. A revolution in morphometrics. *Trends Ecol. Evol.* **8**: 129–132.

- Rohlf, F.J., Loy, A. & Corti, M. 1996. Morphometric analysis of Old World Talpidae (Mammalia, Insectivora) using partial-warp scores. *Syst. Biol.* **45**: 344–362.
- Ruehl, C.B. & DeWitt, T.J. 2005. Trophic plasticity and fine-grained resource variation in populations of western mosquitofish, *Gambusia affinis*. *Evol. Ecol. Res.* **7**: 801–819.
- Rundle, H.D. & Nosil, P. 2005. Ecological speciation. *Ecol. Lett.* **8**: 336–352.
- Sakai, A.K., Allendorf, F.W., Holt, J.S., Lodge, D.M., Molofsky, J., With, K.A., Baughman, S., Cabin, R.J., Cohen, J.E., Ellstrand, N.C., McCauley, D.E., O'Neil, P., Parker, I.M., Thompson, J.N. & Weller, S.G. 2001. The population biology of invasive species. *Annu. Rev. Ecol. Syst.* **32**: 305–332.
- Santiago, C., Gonzalez-Freire, M., Serratos, L., Morate, F.J., Meyer, T., Gomez-Gallego, F. & Lucia, A. 2008. ACTN3 genotype in professional soccer players. *Br. J. Sports Med.* **42**: 72–73.
- Schluter, D. 2000. *The Ecology of Adaptive Radiation*. Oxford University Press, Oxford.
- Schluter, D. 2001. Ecology and the origin of species. *Trends Ecol. Evol.* **16**: 372–380.
- Schluter, D. 2009. The origin of species by natural selection: evidence for ecological speciation and its alternative. *Science* **323**: 737–741.
- Schmidly, D.J. 2002. *Texas Natural History: A Century of Change*. Texas Tech University Press, Lubbock.
- Secor, S.M., Jayne, B.C. & Bennett, A.F. 1992. Locomotor performance and energetic cost of sidewinding by the snake *Crotalus cerastes*. *J. Exp. Biol.* **163**: 1–14.
- Sfakiotakis, M., Lane, D.M. & Davies, J.B.C. 1999. Review of fish swimming modes for aquatic locomotion. *IEEE J. Oceanic Eng.* **24**: 237–252.
- Shibley, B. 1997. Exploratory path analysis with applications in ecology and evolution. *Am. Nat.* **149**: 1113–1138.
- Smith, T.B. & Skúlason, S. 1996. Evolutionary significance of resource polymorphisms in fishes, amphibians, and birds. *Annu. Rev. Ecol. Syst.* **27**: 111–133.
- Smithson, M.J. 2003. *Confidence Intervals*. Sage, Thousand Oaks, CA.
- Sorci, G., Swallow, J.G., Garland, T. & Clobert, J. 1995. Quantitative genetics of locomotor speed and endurance in the lizard *Lacerta vivipara*. *Physiol. Zool.* **68**: 698–720.
- Steinhausen, M.F., Steffensen, J.F. & Andersen, N.G. 2005. Tail beat frequency as a predictor of swimming speed and oxygen consumption of saithe (*Pollachius virens*) and whiting (*Merlangius merlangus*) during forced swimming. *Mar. Biol.* **148**: 197–204.
- Stockwell, C.A. & Weeks, S.C. 1999. Translocations and rapid evolutionary responses in recently established populations of western mosquitofish (*Gambusia affinis*). *Anim. Conserv.* **2**: 103–110.
- Stockwell, C.A., Hendry, A.P. & Kinnison, M.T. 2003. Contemporary evolution meets conservation biology. *Trends Ecol. Evol.* **18**: 94–101.
- Tsuji, J.S., Huey, R.B., Vanberkum, F.H., Garland, T. & Shaw, R.G. 1989. Locomotor performance of hatchling fence lizards (*Sceloporus occidentalis*): quantitative genetics and morphometric correlates. *Evol. Ecol.* **3**: 240–252.
- Vamosi, S.M. 2005. On the role of enemies in divergence and diversification of prey: a review and synthesis. *Can. J. Zool.* **83**: 894–910.
- Van Damme, R., Wilson, R.S., Vanhooydonck, B. & Aerts, P. 2002. Performance constraints in decathletes. *Nature* **415**: 755–756.
- Van Leeuwen, J.L., Lankheet, M.J.M., Akster, H.A. & Osse, J.W.M. 1990. Function of red axial muscles of carp (*Cyprinus carpio*): recruitment and normalized power output during swimming in different modes. *J. Zool.* **220**: 123–145.
- Van Noordwijk, A.J. & de Jong, G. 1986. Acquisition and allocation of resources: their influence on variation in life history tactics. *Am. Nat.* **128**: 137–142.
- Vanhooydonck, B., Van Damme, R. & Aerts, P. 2001. Speed and stamina trade-off in lacertid lizards. *Evolution* **55**: 1040–1048.
- Venables, W. 1975. Calculation of confidence intervals for noncentrality parameters. *J. R. Stat. Soc. Series B Stat. Methodol.* **37**: 406–412.
- Videler, J.J. 1993. *Fish Swimming*. Chapman & Hall, London.
- Videler, J.J. & Wardle, C.S. 1991. Fish swimming stride by stride: speed limits and endurance. *Rev. Fish. Biol. Fish.* **1**: 23–40.
- Vogel, S. 1994. *Life in Moving Fluids*, 2nd edn. Princeton University Press, Princeton, NJ.
- Wainwright, P.C. 1988. Morphology and ecology: functional basis of feeding constraints in Caribbean labrid fishes. *Ecology* **69**: 635–645.
- Wainwright, P.C. 1996. Ecological explanation through functional morphology: the feeding biology of sunfishes. *Ecology* **77**: 1336–1343.
- Wainwright, P.C. 2007. Functional versus morphological diversity in macroevolution. *Annu. Rev. Ecol. Evol. Syst.* **38**: 381–401.
- Wainwright, P.C., Alfaro, M.E., Bolnick, D.I. & Hulsey, C.D. 2005. Many-to-one mapping of form to function: a general principle in organismal design? *Integr. Comp. Biol.* **45**: 256–262.
- Walker, J.A. 1997. Ecological morphology of lacustrine three-spine stickleback *Gasterosteus aculeatus* L. (Gasterosteidae) body shape. *Biol. J. Linn. Soc.* **61**: 3–50.
- Walker, J.A. 2007. A general model of functional constraints on phenotypic evolution. *Am. Nat.* **170**: 681–689.
- Walker, J.A., Ghalambor, C.K., Griset, O.L., McKenney, D. & Reznick, D.N. 2005. Do faster starts increase the probability of evading predators? *Funct. Ecol.* **19**: 808–815.
- Wares, J.P., Hughes, A.R. & Grosberg, R.K. 2005. Mechanisms that drive evolutionary change: insights from species introductions and invasions. In: *Species Invasions. Insights into Ecology, Evolution, and Biogeography* (D.F. Sax, J.J. Stachowicz & S.D. Gaines, eds), pp. 229–258. Sinauer Associates, Inc., Sunderland, MA.
- Webb, P.W. 1975. Hydrodynamics and energetics of fish propulsion. *Bull. Fish. Res. Board Can.* **190**: 1–159.
- Webb, P.W. 1982. Locomotor patterns in the evolution of Actinopterygian fishes. *Am. Zool.* **22**: 329–342.
- Webb, P.W. 1983. Speed, acceleration and manoeuvrability of two teleost fishes. *J. Exp. Biol.* **102**: 115–122.
- Webb, P.W. 1984. Body form, locomotion, and foraging in aquatic vertebrates. *Am. Zool.* **24**: 107–120.
- Webb, P.W. 1986a. Effect of body form and response threshold on the vulnerability of four species of teleost prey attacked by largemouth bass (*Micropterus salmoides*). *Can. J. Fish. Aquat. Sci.* **43**: 763–771.
- Webb, P.W. 1986b. Locomotion and predator-prey relationships. In: *Predator-Prey Relationships* (G.V. Lauder & M.E. Feder, eds), pp. 24–41. University of Chicago Press, Chicago, IL.

- Webb, P.W. 1988. Steady swimming kinematics of tiger musky, an esociform accelerator, and rainbow trout, a generalist cruiser. *J. Exp. Biol.* **138**: 51–69.
- Webb, P.W. 1998. Swimming. In: *The Physiology of Fishes* (D.H. Evans, ed.), pp. 3–24. CRC Press, Boca Raton, FL.
- Webb, C. & Joss, J. 1997. Does predation by the fish *Gambusia holbrooki* (Atheriniformes: Poeciliidae) contribute to declining frog populations? *Aust. Zool.* **30**: 316–324.
- Weihhs, D. 1973. The mechanism of rapid starting of slender fish. *Biorheology* **10**: 343–350.
- Weihhs, D. 1989. Design features and mechanics of axial locomotion in fish. *Am. Zool.* **29**: 151–160.
- Weihhs, D. & Webb, P.W. 1983. Optimization of locomotion. In: *Fish Biomechanics* (P.W. Webb & D. Weihhs, eds), pp. 339–371. Praeger, New York.
- Whitlock, M.C. 2005. Combining probability from independent tests: the weighted Z-method is superior to Fisher's approach. *J. Evol. Biol.* **18**: 1368–1373.
- Williams, G.C. 1992. *Natural Selection: Domains, Levels and Challenges*. Oxford University Press, Oxford.
- Wilson, R.S., James, R.S. & Van Damme, R. 2002. Trade-offs between speed and endurance in the frog *Xenopus laevis*: a multi-level approach. *J. Exp. Biol.* **205**: 1145–1152.
- Wilson, R.S., James, R.S., Kohlsdorf, T. & Cox, V.M. 2004. Interindividual variation of isolated muscle performance and fibre-type composition in the toad *Bufo viridus*. *J. Comp. Physiol. B, Biochem. Syst. Environ. Physiol.* **174**: 453–459.
- Winkelman, D.L. & Aho, J.M. 1993. Direct and indirect effects of predation on mosquitofish behavior and survival. *Oecologia* **96**: 300–303.
- Wright, S. 1934. The method of path coefficients. *Ann. Math. Stat.* **5**: 161–215.
- Wu, T.Y. 1971. Hydromechanics of swimming propulsion. Part 1. Swimming of a two-dimensional flexible plate at variable forward speeds in an inviscid fluid. *J. Fluid Mech.* **46**: 337–355.
- Yang, N., MacArthur, D.G., Gulbin, J.P., Hahn, A.G., Beggs, A.H., Easteal, S. & North, K. 2003. ACTN3 genotype is associated with human elite athletic performance. *Am. J. Hum. Genet.* **73**: 627–631.
- Zelditch, M.L., Swiderski, D.L., Sheets, H.D. & Fink, W.L. 2004. *Geometric Morphometrics for Biologists: A Primer*. Elsevier Academic Press, London.
- Zou, G.Y. 2007. Exact confidence interval for Cohen's effect size is readily available. *Stat. Med.* **26**: 3054–3056.

Supporting information

Additional Supporting Information may be found in the online version of this article:

Table S1 Pearson correlation coefficients between kinematic variables and the divergence vector derived from the predator regime term of the MANCOVA (positive end of axis associated with high-predation populations).

Table S2 Principal components analysis of morphological and kinematic variables.

Appendix S1 Methodological details.

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Received 14 August 2008; revised 29 January 2009; accepted 2 February 2009

Supporting Information

Tradeoff between steady and unsteady swimming underlies predator-driven divergence in *Gambusia affinis*

R. Brian Langerhans

Appendix S1. Methodological Details

Streamlining Index

Using lateral and ventral images of specimens, I calculated the depth and width of each fish body at 100 equally spaced positions along the midline using MorphoSys software version 1.29 (Meacham, 1993). I then calculated the depth and width at the same 100 positions along the midline for a streamlined body (foil drawn from the U.S. National Advisory Committee for Aeronautics, NACA) having the same maximum thickness as the fish. I accomplished this using the equation for NACA 0000-series foils without camber (Jacobs *et al.*, 1933):

$$t_{\text{NACA}}(x) = 2 \left(t_{\text{max}}/0.2 (0.2969\sqrt{x} - 0.1260x - 0.3516x^2 + 0.2843x^3 - 0.1015x^4) \right)$$

where t_{max} is the maximum thickness measured along the fish's midline, and x is the position along the midline given in proportional units (distance from snout tip / body length). This equation was employed twice for each fish: once for calculation of depth values and once for calculation of width values. To compare a fish's volume distribution to that of a NACA-streamlined body, the ratio of second moments of area was used ($I_{\text{fish}} / I_{\text{NACA}}$). This procedure integrates the product of many small volumes along a body by their squared distance from an axis of rotation. Following McHenry & Lauder (2006), the axis of rotation was positioned anterior to the rostrum in a dorsoventral direction; the second moment of area was calculated from the depth and width values using equation 4 in McHenry & Lauder (2006). This streamlining ratio ($\text{SR} = I_{\text{fish}} / I_{\text{NACA}}$) describes fish bodies that can range from shapes having more anterior volume relative to a foil (values < 1) to shapes having more posterior volume relative to a foil (values > 1), with the value 1.0 representing a perfect match in volume distribution between a fish's body form and the foil. Because SR values can range from less than to greater than 1.0, a hump-shaped relationship is predicted with steady-swimming performance (i.e. highest performance at 1.0, reduced performance either less than or greater than 1.0; see Langerhans & Reznick, in press for empirical confirmation in *Gambusia*). To approximately linearize this predicted relationship, I calculated a streamlining index: $\text{SI} = 1 - |1 - \text{SR}|$. SI values ignore the specific nature of how a body deviates from the optimum, and can range from $-\infty$ to 1.0, with 1.0 representing a perfect match in volume distribution between a fish's body and the NACA-streamlined body. The direction of deviation from the optimum is not of interest here, as drag costs are expected to be approximately symmetric about the optimum SR across the relatively small phenotypic range observed within species (Langerhans & Reznick, in press). Because *G. affinis* individuals exhibited SR values on either side of the predicted optimum, I used SI in all statistical analyses.

Steady-swimming Kinematics

Tail-beat frequency (f , Hz) was measured from the video as the inverse of the average period (sec) of ten complete tail-beat cycles. I measured the caudal fin trailing-edge depth (B , mm) as the vertical distance between the dorsal-most and ventral-most points on the caudal fin.

For all other measured kinematic parameters, I performed frame-by-frame analysis of a video segment of steady swimming spanning three complete tail beats. Landmarks were digitized from video frames using tpsDig software (Rohlf, 2006). Rostral amplitude (R , mm) was measured as half of the average distance between right and left excursions of the anterior tip of the rostrum. I tracked the tip of the caudal fin to measure tail-beat amplitude (H , mm) as half of the average distance between right and left excursions of the fin. I estimated propulsive wavelength (λ , mm) by doubling the posterior half-wavelength (see Webb *et al.*, 1984; Webb *et al.*, 1992). Because these latter three parameters represent averages across three tail beats, I calculated the repeatability of these measurements across the three tail beats using the intraclass correlation coefficient from a model II ANOVA (Lessells & Boag, 1987; Sokal & Rohlf, 1995). All estimates were highly repeatable (H : intraclass correlation coefficient, $r = 0.68$, $P < 0.0001$; λ : $r = 0.79$, $P < 0.0001$; R : $r = 0.52$, $P < 0.0001$).

Lighthill's elongated-body theory (Lighthill, 1975; Wu, 1977; Videler, 1993) indicates that the mean thrust generated during undulatory swimming can be calculated from conditions at the trailing edge of the caudal fin. Here I used elongated-body theory to estimate power, or the mechanical rate of working, as $P \propto f^2 H^2 B^2 (1 - U/c)$. This parameter can be thought of as the total lateral force produced by the tail to overcome drag forces and maintain constant-velocity swimming. Holding swimming speed constant, as I did in this study, a lower P indicates greater overall locomotor efficiency (i.e. less power consumed to swim at a given speed, analogous to miles per gallon).

Table S1. Pearson correlation coefficients between kinematic variables and the divergence vector derived from the predator regime term of the MANCOVA (positive end of axis associated with high-predation populations). Variables exhibiting a correlation $\geq |0.6|$ are in bold text.

Variable	d
Tail-beat frequency (f)	-0.29
Rostral amplitude (R/L)	+0.81
Tail-beat amplitude (H/L)	+0.79
Propulsive wavelength (λ/L)	-0.61
Propulsive wave speed (c/L)	-0.65

Table S2. Principal components analysis of morphological and kinematic variables. Lateral body shape is represented by the divergence vector derived from the predator regime term in MANCOVA (positive end of the axis describes fish with smaller heads and larger caudal peduncles). Factor loadings in bold indicate variables that load strongly on each axis (loadings $\geq |0.6|$). Only axes retained for analysis are shown (retention of axes according to broken-stick criterion).

Data Type	Variable	PC1	PC2	PC3
Morphology	body length ($\log_{10} L$)	+0.85	-0.21	
	lateral body shape (d)	-0.13	-0.87	
	streamlining index ($\arcsin \sqrt{SI}$)	+0.77	+0.20	
	caudal fin depth (B/L)	+0.27	+0.53	
	proportion of variance	0.35	0.28	
Kinematics	tail-beat frequency (f)	+0.70	-0.02	+0.71
	rostral amplitude (R/L)	-0.80	+0.43	+0.23
	tail-beat amplitude (H/L)	-0.69	+0.52	+0.40
	propulsive wavelength (λ/L)	+0.40	+0.74	-0.53
	propulsive wave speed (c/L)	+0.84	+0.50	+0.21
	proportion of variance	0.49	0.25	0.21

Supporting Information – References

- Jacobs, E.N., Ward, K.E. & Pinkerton, R.M. 1933. The characteristics of 78 related airfoil sections from tests in the variable-density wind tunnel. *NACA rep. 460*: 299-354.
- Langerhans, R.B. & Reznick, D.N. In Press. Ecology and evolution of swimming performance in fishes: predicting evolution with biomechanics. In: *Fish Locomotion: An Etho-ecological Perspective* (P. Domenici & B. G. Kapoor, eds). Science Publishers, Enfield.
- Lessells, C.M. & Boag, P.T. 1987. Unrepeatable repeatabilities: a common mistake. *Auk* **104**: 116-121.
- Lighthill, M.J. 1975. *Mathematical Biofluidynamics*. Society for Applied and Industrial Mathematics, Philadelphia.
- McHenry, M.J. & Lauder, G.V. 2006. Ontogeny of form and function: locomotor morphology and drag in zebrafish (*Danio rerio*). *J. Morphol.* **267**: 1099-1109.
- Meacham, C.A. 1993. MorphoSys: an interactive machine vision program for acquisition of morphometric data. In: *Advances in Computer Methods for Systematic Biology. Artificial Intelligence, Databases, Computer Vision*. (R. Fortuner, ed., pp. 393-402. Johns Hopkins University Press, Baltimore, M.D.
- Rohlf, F.J. 2006. TpsDig, Department of Ecology and Evolution, State Univ. New York. Stony Brook.
- Sokal, R.R. & Rohlf, F.J. 1995. *Biometry*, 3rd edn. Freeman, NY.
- Videler, J.J. 1993. *Fish Swimming*. Chapman & Hall, London.
- Webb, P.W., KostECKI, P.T. & Stevens, E.D. 1984. The effect of size and swimming speed on locomotor kinematics of rainbow trout. *J. Exp. Biol.* **109**: 77-95.
- Webb, P.W., Hardy, D.H. & Mehl, V.L. 1992. The effect of armored skin on the swimming of longnose gar, *Lepisosteus osseus*. *Can. J. Zool.* **70**: 1173-1179.
- Wu, T.Y. 1977. Introduction to the scaling of aquatic animal locomotion. In: *Scale Effects in Animal Locomotion* (T. J. Pedley, ed., pp. 203-232. Academic Press, New York.