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Morphology, performance, fitness: functional insight into a post-Pleistocene radiation of mosquitofish

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Bahamas mosquitofish (*Gambusia hubbsi*) colonized blue holes during the past approximately 15 000 years and exhibit relatively larger caudal regions in blue holes that contain piscivorous fish. It is hypothesized that larger caudal regions enhance fast-start escape performance and thus reflect an adaptation for avoiding predation. Here I test this hypothesis using a three-pronged, experimental approach. First, *G. hubbsi* from blue holes with predators were found to possess both greater fast-start performance and greater survivorship in the presence of predatory fish. Second, using individual-level data to investigate the morphology–performance–fitness pathway, I found that (i) fish with larger caudal regions produced higher fast-start performance and (ii) fish with higher fast-start performance enjoyed greater survivorship in the presence of fish predators—trends consistently observed across both predator regimes. Finally, I found that morphological divergence between predator regimes at least partially reflects genetic differentiation, as differences were retained in fish raised in a common laboratory environment. These results suggest that natural selection favours increased fast-start performance in the presence of piscivorous fish, consequently driving the evolution of larger caudal regions. Combined with previous work, this provides functional insight into body shape divergence and ecological speciation among Bahamian blue holes.

Keywords: adaptation; biomechanics; locomotion; morphology; population differentiation; predation

1. INTRODUCTION

Animals use diverse means of avoiding predation (reviewed in Langerhans 2006). For most fish, the fast-start escape response is the primary mechanism used to evade predator strikes (Domenici in press). The fast start is a rapid, high-energy swimming burst elicited from threatening stimuli. Because predation varies across space and time, divergent selection on locomotor abilities between predator regimes may be a major factor in morphological evolution and speciation in fishes (Webb 1984; Langerhans *et al.* 2007).

Theory and recent empirical work suggests that larger caudal regions (i.e. posteriorly large lateral surface area) should enhance fast-start performance (Webb 1984; Langerhans *et al.* 2004; Domenici *et al.* 2008; Tytell & Lauder 2008). This long-standing purported link between morphology and locomotion has seldom been tested in detail—no prior study has used individual-level data to test this hypothesis using high-speed video data. Moreover, it has long been suggested that greater fast-start performance enhances the probability of survival with predators, although this has very rarely been tested (Webb 1986; Katzir & Camhi 1993; Walker *et al.* 2005). If these links between morphology, performance and fitness are accurate, then a clear evolutionary prediction exists: fish experiencing high levels of predation from piscivorous fish will evolve larger caudal regions.

Consistent with this prediction, recent work uncovered that Bahamas mosquitofish (*Gambusia hubbsi*) inhabiting inland blue holes (water-filled, vertical caves) with predatory fish (*Gobiomorus dormitor*) exhibit larger caudal regions than populations in blue holes without piscivorous fish (Langerhans *et al.* 2007). Whether this post-Pleistocene radiation of mosquitofish actually involves selection on fast-start performance has not yet been tested. If enlarged caudal regions reflect an antipredator adaptation, three predictions should be upheld: (i) *G. hubbsi* from blue holes with piscivorous fish (H) should exhibit greater fast-start performance and greater survivorship in the presence of predators than *G. hubbsi* from blue holes without fish predators (L); (ii) body shape *per se* (not other traits covarying with the predator regime) should confer greater fast-start performance and consequently greater survivorship in the presence of predatory fish; and (iii) body shape divergence should at least partially reflect genetically based differentiation. To test the first prediction, I compare fast-start performance and survival with predators among fish from different predator regimes. I test the second prediction by measuring the morphology–performance–fitness (M–P–F) pathway and calculating selection on body shape derived solely from selection on fast-start performance—this provides the first direct test of this long-hypothesized M–P–F pathway. Finally, I use a common-garden experiment to test the third prediction.

2. MATERIAL AND METHODS

Fish from four blue holes (2 L, 2 H) were photographed alive for morphometric analysis following Langerhans *et al.* (2007). Body size was estimated as centroid size; body shape was calculated by assigning each fish a score on a canonical axis describing lateral body shape variation. This axis ranges from shapes characteristic of L blue holes (small caudal region) to those characteristic of H blue holes (large caudal region) (electronic supplementary material, figure S1).

Fast-start performance trials were recorded with a high-speed digital video camera (electronic supplementary material). For each fast-start video sequence (40 ms), I measured four performance variables: d_{net}, $\bar{\omega}_{\text{S1}}$, $v_{\max}$ and $a_{\max} \cdot d_{\text{net}}$ is the net distance travelled by the centre of mass. $\bar{\omega}_{\text{S1}}$ is the average rotational velocity of the head during stage 1 of the fast start (stage 1 rotation angle divided by stage 1 duration). $v_{\max}$ and $a_{\max}$ are the maximum velocity and acceleration, computed using the mean-squared error quintic spline to smooth the centre-of-mass displacement data. All four variables have been previously implicated as important in evading predatory strikes (Walker *et al.* 2005). Differences between predator regimes in fast-start performance were tested using nested multivariate analysis of covariance, followed by mixed-model nested analysis of covariance with each performance variable. Predator regime and population

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nested within predator regime served as independent variables, and centroid size served as a covariate.

Predation trials were conducted in large experimental tanks (440 l), in which four adult *G. hubbsi* (one of each sex from two populations having different predator regime statuses) were exposed to one *G. dormitor* (electronic supplementary material). All *G. hubbsi* used in the experiment had previously (within 48 h) been photographed for morphometrics and had their fast-start performance measured. At the conclusion of each trial, *G. hubbsi* survivors were removed and identified using photographs. I used Wilcoxon signed-rank test to examine whether H fish exhibited greater survivorship than L fish.

I investigated the M–P–F pathway using a two-step process. First, effects of M (centroid size and body shape) on P (four performance variables) were examined using (multiple) regression to calculate standardized performance gradients and test significance (Arnold 1983). The effects of P on F (survival during predation trials) were examined using (multiple) regression to calculate standardized fitness gradients (Arnold 1983; Lande & Arnold 1983) and (multiple) logistic regression to test significance. I used model selection (Akaike Information Criterion, AIC; Akaike 1992) to determine the best set of independent variables for adequately predicting each dependent variable (electronic supplementary material). In all cases, I employ one-tailed p-values for tests with *a priori* predictions.

I conducted a common-garden experiment to test whether body shape differences between predator regimes reflected genetic differentiation (electronic supplementary material). I reared laboratory-born fish (F1 and F2) in a 120 l recirculating system and then photographed each fish for morphometric analysis. To test whether differences observed in the wild were maintained after laboratory rearing, I used a discriminant function derived from wild-caught fish to assign each laboratory-born fish to a predator regime. Significance was tested using a binomial test based on whether each fish was correctly assigned to its predator regime of origin.

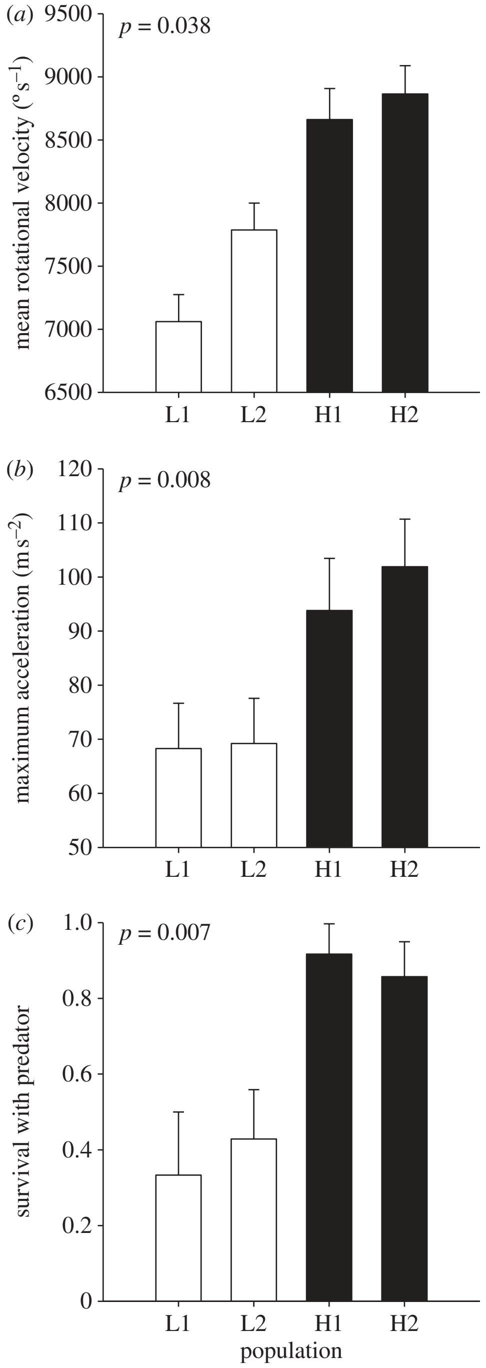

Figure 1. Variation among *G. hubbsi* populations in (*a*) $\bar{\omega}_{S1}$, (*b*) a_{max} and (*c*) proportion surviving with a fish predator (least-squares means ± 1 s.e.). Open bars indicate low-predation blue holes; filled bars indicate high-predation blue holes. One-tailed significance is denoted in each graph.

3. RESULTS

Gambusia hubbsi from H blue holes exhibited greater fast-start performance (18% higher $\bar{\omega}_{S1}$, 42% higher a_{max}, on average) and higher survivorship in the presence of predatory fish (50% higher survival) than conspecifics from L blue holes (figure 1). No differences were observed for the other two fast-start performance variables (electronic supplementary material, table S1).

Analysis of the M–P–F pathway indicates that body shape differences between predator regimes, consequently facilitating locomotor differences, are largely responsible for differences in survivorship (figure 2; electronic supplementary material, tables S2 and S3). Three M–P relationships were strong and highly significant (all one-tailed $p < 0.0001$): smaller fish produced greater average rotational velocity, and fish with larger caudal regions had both greater average rotational velocity and maximum acceleration. One M–P relationship approached significance: fish with larger caudal regions tended to generate greater maximum velocity (one-tailed $p = 0.09$). One M–P relationship was not significant, but suggestive based on AIC: smaller fish tended to generate greater maximum acceleration ($p = 0.18$). Two P–F relationships were strong and highly significant: fish with greater average rotational velocity and maximum acceleration exhibited higher survival (both one-tailed $p < 0.008$). All relationships were consistently observed within predator regimes, indicating that correlated traits that merely covary with the predator regime cannot explain these findings (electronic supplementary material). The total selection gradient on body shape—selection resulting exclusively from its influence on survival as mediated by its effects on fast-start performance—was $\beta = 0.28$, meaning that a positive change in one standard deviation of the body shape axis is predicted to result in 28 per cent greater survival probability. Because H fish exhibit a body shape axis score 1.63 standard deviations greater, on average, than L fish, they are predicted to enjoy an approximately 46 per cent greater survival probability. Results from predation trials are remarkably close to this prediction, as H fish exhibited 50 per cent greater survivorship than L fish.

After rearing in a common laboratory environment, 80 per cent of the laboratory-born fish were correctly assigned to their predator regime of origin using a discriminant function derived from wild fish ($p < 0.0001$;

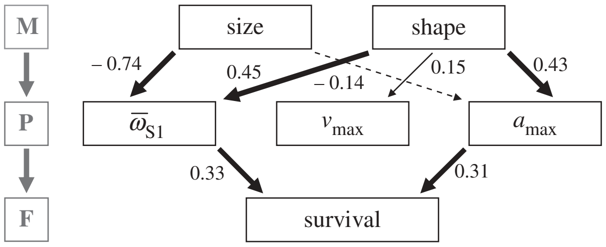

Figure 2. M–P–F pathway for *G. hubbsi* in the presence of predatory fish. Path coefficients represent standardized performance (M–P) and fitness gradients (P–F). Paths selected using AIC, line thickness reflects the strength of the path, solid lines represent positive effects and dashed lines represent negative effects. Size: centroid size; shape: lateral shape axis (ranging from small to large caudal regions); other abbreviations follow the text.

electronic supplementary material). This suggests that body shape differences between predator regimes observed in the wild at least partially reflect genetic differentiation.

4. DISCUSSION

Fish from H blue holes, having larger caudal regions, exhibited higher fast-start performance and survival with predators than L fish, strongly suggesting adaptive differentiation; however, this does not reveal causation. A powerful approach to testing the adaptive significance of morphological traits is to examine the M–P–F pathway (Arnold 1983). Although rarely investigated, M–P–F pathways can offer a strong, functional understanding of how selection acts on morphology. Here, I found that fish with larger caudal regions produced greater fast-start performance and consequently experienced higher survivorship with predators. Differences in survivorship between predator regimes could largely be accounted for by selection on body shape, matching *a priori* predictions.

Fish with larger caudal regions presumably generated greater acceleration and rotational velocities during fast starts owing to higher thrust produced by the larger surface area, and higher muscle power (e.g. greater white muscle mass), respectively (Domenici *et al.* 2008). Higher acceleration and rotational velocity probably increased survivorship by generating more rapid turns away from danger, increasing evasion success. Interestingly, body size was under strong selection in the presence of predators (total selection, $\beta = -0.29$). However, only body shape, not size, is known to differ between predator regimes in *G. hubbsi*. This suggests that selection on fast-start performance might explain body shape divergence, but other factors are important for body size evolution in blue holes. Moreover, traits other than body morphology probably influence fast-start performance (e.g. median fins, muscle architecture), and traits other than fast-start performance probably influence survival with predators (e.g. behavioural avoidance of predators). Yet, results here suggest that body morphology and fast-start performance represent major targets of selection in the presence of predators, accurately predicting observed survivorship differences between fish from divergent predator regimes.

Predation is a major force of phenotypic evolution and speciation. This study suggests that natural selection via predation by piscivorous fish has driven the evolution of larger caudal regions, greater fast-start performance and higher survivorship in *G. hubbsi* inhabiting blue holes with predatory fish. Based on theory and recent empirical work in a congener (e.g. Langerhans in press), enlarged caudal regions are predicted to suffer endurance costs during steady swimming, and perhaps explain why fish in L blue holes—where cruising for food and mates, not bursting from predators, is commonplace—exhibit smaller caudal regions. Future work should test this 'flip-side' to the M–P–F pathway examined here. In any case, morphological divergence between blue holes has apparently played an important role in the process of ecological speciation. First, fish inhabiting divergent predator regimes exhibit divergent body shapes and consequently have reduced mating probabilities owing to assortative mating for body shape (Langerhans *et al.* 2007). Second, if L fish were to colonize H blue holes, they would probably suffer increased mortality relative to resident fish (this study). Both processes increase reproductive isolation between fish from different predator regimes relative to fish from the same predator regime (i.e. ecological speciation).

Experiments were approved by the Washington University Animal Studies Committee and the Bahamas Department of Fisheries.

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Electronic Supplementary Material

R. B. Langerhans, "Morphology, performance, fitness: functional insight into a post-Pleistocene radiation of mosquitofish"

MATERIAL AND METHODS

Gambusia hubbsi were collected from four inland blue holes on Andros Island, the Bahamas (L1, L2, H1, H2 from Langerhans *et al.* 2007). In low-predation (L) blue holes *G. hubbsi* experience a relatively predator-free environment devoid of any piscivorous fish, while in high-predation (H) blue holes they coexist with the highly piscivorous bigmouth sleeper (*Gobiomorus dormitor*), a major predator of mosquitofish (McKaye *et al.* 1979; Winemiller and Ponwith 1998; Bacheler *et al.* 2004; Langerhans *et al.* 2007; R. B. Langerhans, unpubl. data). For morphometric analysis, I digitized ten landmarks on each image using tpsDig (Rohlf 2006), and used geometric morphometric methods to evaluate body shape variation (see Langerhans *et al.* 2007 for details). 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. Shape variables (partial warps and uniform components) were derived from landmark data using Generalized Procrustes Analysis in tpsRegr (Rohlf 2005). These variables were used in assigning each fish a score on a body shape axis derived in Langerhans *et al.* (2007) describing shape variation between predator regimes in the four blue holes examined in this study. The shape axis primarily describes variation in the size of the caudal peduncle region (see Fig. S1). Because these fish cannot gape limit their primary predator in blue holes (*G. dormitor*), it has been suggested that observed body shape differences are related to locomotor performance, where selection favors increased fast-start performance in the presence of predators (Langerhans *et al.* 2007).

Fish used in locomotor-performance and predation trials were collected from the wild and held in the laboratory at least 48 hours prior to experimentation (mean ± std. err, males: 22.8 ± 0.4 mm SL; females: 26.5 ± 0.7 mm SL). Fast-start performance trials were initiated by transferring a single individual to a 25 × 15 cm staging arena with a 25-mm square grid affixed to the bottom. The staging arena was evenly illuminated and all sides were opaque. A 75-L water bath and ice packs served to maintain a relatively constant water temperature (24.6 ± 0.1°C). Fast starts were recorded from above using a high-speed digital video camera (Photron Fastcam PCI R2; San Diego, CA) set to 500 frames s^{-1} and 512 × 240 pixel resolution. To limit vertical displacement of fish during escape responses, water depth was maintained at 2 cm (~3 times the average body depth). Fish were allowed to acclimate for 10 min before stimulated to perform an escape response. I elicited a fast-start response by startling the fish with a sudden, downward thrust of a cylindrical wooden probe (5 mm diameter, 585 mm length), hitting the bottom of the stage within 3 cm of the fish. To minimize possible wall effects, I only examined trials where the fish was free from any apparent contact with the surface, bottom, or sides. Multiple fast-starts were examined for each fish (3-5), and maximal values of performance variables were retained for analyses. The number of fast-starts examined for each fish did not differ between predator regimes ($P = 0.59$; L: 3.29 ± 0.32, H: 3.59 ± 0.34). For each fast-start sequence, I digitized the center of mass of the fish from the frame prior to the initial head movement to the 20th frame following initial head movement using tpsDig (i.e., each sequence comprised 21 frames). Measurement error in displacement data resulting from the visual estimation of the center of mass was calculated by digitizing two fast-start video sequences three times each, and incorporated into the mean-square error quintic spline estimation of velocity and acceleration (Walker 1998). While I included four commonly-measured components of the fast-start escape sequence, other variables have also been previously suggested as important in predator-prey encounters, such as the distance from the predator at initiation of response, escape angle, overall responsiveness, and latency of response (Walker *et al.* 2005; Fuiman *et al.* 2006). These latter variables were not measured here because I was specifically interested in fast-start components that might be largely determined by morphological variation, while these variables are likely determined by other mechanisms, mainly related to neural control. Moreover, most of these variables would have been difficult or impossible to measure here given the experimental design (e.g., predators were not present during trials, and thus measurements relative to the predator's location or trajectory were not possible). Repeatability (i.e., intraclass correlation coefficient, Lessells & Boag 1987) of each performance variable was highly significant (all $P < 0.0001$, mean $r = 0.57$). When testing for differences in fast-start performance between predator regimes, a term for sex was initially included in all models, but was excluded from all final analyses because it was never significant (all $P > 0.25$).

In predation trials ($n = 13$), experimental tanks were devoid of any potential source of refuge to minimize the role of behaviors other than fast-starts in avoiding predation. Alternative behavioral means of avoiding predation might obviously be important in the wild, but here I wished to focus exclusively on the importance of fast-start performance in predator-prey interactions. The number of *G. hubbsi* included in each trial (i.e., 4) was primarily selected to ensure easy identification of individuals without the need for individual tags (which could influence performance and survival). When *G. hubbsi* were initially placed in tanks, *G. dormitor* was temporarily removed to allow 30-min acclimation. Trials were monitored every two hours during daylight (not monitored at night), and halted when at least half of the fish had been consumed by the predator. Eight trials occurred overnight, while the remaining trials were completed before dusk (7.8 ± 1.3 hrs). The number of fish consumed in a given trial was not significantly associated with trial duration ($r = 0.48$, $P = 0.10$). Survivors were removed at the completion of each trial, and easily identified using photographs. *Gobiomorus dormitor* were collected from the two blue holes with predators (H1, H2). Nine *G. dormitor* were used during the experiment, ranging in size from 108 to 136 mm SL (119.7 ± 3.7 mm SL). There were no effects of the identity ($F_{8,4} = 0.31$, $P = 0.93$) or size ($r = -0.44$, $P = 0.13$) of *G. dormitor* on the number of *G. hubbsi* consumed within a trial. There were no differences between sexes in survivorship of *G. hubbsi* (Wilcoxon signed-ranks, $P = 1.0$).

For the analysis of the M-P-F pathway, standardized performance gradients were calculated as the standardized (partial) regression coefficient of M on P (change in standard deviation units of a performance variable caused by the change of one standard deviation of a morphological variable). Standardized fitness gradients were calculated as the (partial) regression coefficient of P on F, where performance traits are standardized within trials and relative fitness (survival divided by mean fitness) is calculated within trials (proportional change in survival probability caused by change of one standard deviation in performance variable; Lande & Arnold 1983). Selection gradients on morphology can be partitioned into pathways to fitness (i.e., through different performance variables), and calculated as the product of the performance gradient and the fitness gradient (Arnold 1983). I calculated the total selection gradient for body shape, resulting from its influence on fitness as mediated exclusively by its affects on fast-start performance, as the sum of the products of performance gradients and fitness gradients along each path from body shape to survival. In model selection, model sets included all possible combinations of independent variables. Because I found no evidence for nonlinear relationships (squared terms), models included only linear terms. I used AIC*c* (AIC corrected for sample size) for the examination of P-F relationships because the number of predictor variables typically exceeded n/40 (Johnson & Omland 2004). Selected models exhibited the lowest AIC and highest Akaike weight (probability that a particular model is the best model given the candidate set of models) (Burnham & Anderson 2002). When model selection was ambiguous based on this criteria (i.e., highly similar AIC values or Akaike weights), I further examined statistical significance of predictor variables, and only included terms with P-values < 0.2. To assess the consistency of M-P-F results across predator regimes, I performed three additional procedures: 1) examined interaction terms for predator regime × predictor variables, 2) assessed whether the sign of relationships were consistent within each predator regime, and 3) used the weighted Z-transform test (weighted by the reciprocal of the squared standard error; Whitlock 2005) to combine probabilities of relationships across analyses performed within predator regimes.

Although previous work suggests that body shape differences between predator regimes likely have a genetic basis—morphological differences between *Gambusia* species, and between populations within *Gambusia* species, often exhibit a strong genetic basis (see references in Langerhans *et al.* 2007)—this hypothesis has not yet been tested for blue hole populations of *G. hubbsi*. While enlarged caudal regions in the presence of predators might be induced (i.e., phenotypic plasticity), and still reflect an antipredator adaptation, selection is not expected to favor plasticity in this system as blue holes appear strongly isolated (Schug *et al.* 1998, Langerhans *et al.* 2007), offering little to no advantage to plasticity relative to constitutive defenses (DeWitt & Scheiner 2004). However, predator-induced plasticity might often play a significant role in predator-prey interactions in other fish systems (e.g., Brönmark & Miner 1992, Eklöv & Jonsson 2007, Januszkiewicz & Robinson 2007, Chivers *et al.* 2008), and its importance in this system is currently unknown. In the common-garden experiment testing for genetically-based morphological differentiation, wild-caught females were housed in the laboratory for at least one month prior to delivering offspring in an effort to minimize potential maternal effects associated with natal environments. All fish were raised to adulthood in 3-L tanks within a 120-L re-circulating system (Aquatic Habitats 2-shelf benchtop system; Apopka, FL), maintained at 25°C with a 14L:10D photoperiod. First-generation (F1) offspring were acquired from three wild-caught females from each of the four blue holes examined in the previous experiments. Second-generation (F2) offspring were acquired from two F1 females from each of two populations (L1, H2). Fish densities were similar for all populations (0.58 ± 0.12 fish per liter), and did not differ between predator regimes (t-test, $P = 0.88$). Fish were fed newly hatched brine shrimp nauplii daily until 8 weeks of age when fish began receiving frozen daphnia and bloodworms. Fish were photographed after approximately 30 weeks of rearing, and ten landmarks were digitized on each image following Langerhans *et al.* (2007).

RESULTS

MANCOVA revealed significant differences between predator regimes in fast-start performance ($F_{4,69} = 10.54$, $P < 0.0001$). Examination of the canonical axis derived from the predator regime term of the model suggested that differences were primarily evident for $\overline{\omega}_{S1}$ and $a_{\max}$. These findings were confirmed using mixed-model nested ANCOVAs for each performance variable (table S1). It is unclear why d_{net} and $v_{\max}$ exhibited no differences between predator regimes, nor exhibited any influence on survival. One possibility is that estimates using an artificial stimulus might not provide accurate estimations of these variables during actual predatory encounters. However, it is unlikely that increased measurement error is responsible for this finding, as these two variables are not expected to exhibit any greater measurement error than the other two traits, especially $a_{\max}$, which represents a second derivative.

When evaluating the consistency of M-P-F results across predator regimes, I found no evidence of inconsistency for any relationship. 1) In all cases, interactions between predator regime and predictor variables were nonsignificant (all $P > 0.17$ for M-P; all $P > 0.68$ for P-F), and inclusion of the terms for predator regime and its interactions did not affect the significance of predictor variables; 2) sign of all relationships were consistent when examined separately within each predator regime; 3) combining probabilities using the weighted Z-transform test revealed highly consistent results, as no qualitative change occurred in the significance for any relationship.

Fish raised in the common-garden experiment were assigned to a predator regime based on body shape (geometric shape variables, partial warps and uniform components) using a discriminant function derived from wild-caught fish (i.e., those used in the experiments). Overall, 44 of 55 fish were correctly assigned to their predator regime of origin ($P < 0.0001$). For F1 fish, 31 of 38 were correctly assigned ($P = 0.0001$), while 13 of 17 F2 fish were correctly assigned ($P = 0.049$).

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Table S1. Mixed-model nested analysis of covariance (ANCOVA) results examining variation in fast-start performance among *Gambusia hubbsi* populations. *P*-values for the predator regime term are one-tailed because *a priori* predictions exist for all variables.

	Predator Regime	Pop (Predator Regime)	Centroid Size
Performance Variable	*P*	*P*	*P*
Net distance travelled (d_{net})	0.4338	0.0005	0.6717
Mean rotational velocity ($\overline{\omega}_{\mathrm{S1}}$)	0.0383	0.0521	< 0.0001
Maximum Velocity (v_{max})	0.3657	0.0241	0.3594
Maximum Acceleration (a_{max})	0.0083	0.8232	0.5861

Table S2. Summary of model selection statistics evaluating relationships between body morphology and fast-start performance in *Gambusia hubbsi*. Models are ordered from best to worst. Performance abbreviations follow the main text. Bold text indicates the selected model. For d_{net}, no model was selected as all terms in all models were nonsignificant (all $P > 0.62$). For v_{max}, the model with only body shape was selected based on AIC and because centroid size was nonsignificant in all models (all $P > 0.45$). For a_{max}, two models were virtually indistinguishable; I selected the larger model because centroid size exhibited a low P value in the model ($P = 0.18$).

Dependent Variable	Model	AIC	ΔAIC	Akaike weight
d_{net}	centroid size	267.51	0.00	0.43
	body shape	267.66	0.15	0.40
	centroid size + body shape	269.41	1.89	0.17
$\overline{\omega}_{\text{S1}}$	**centroid size + body shape**	**1062.59**	**0.00**	**1.00**
	centroid size	1094.53	31.95	0.00
	body shape	1132.02	69.44	0.00
v_{max}	**body shape**	**454.94**	**0.00**	**0.51**
	centroid size	456.20	1.26	0.27
	centroid size + body shape	456.63	1.69	0.22
a_{max}	body shape	558.38	0.00	0.52
	centroid size + body shape	**558.53**	**0.15**	**0.48**
	centroid size	571.73	13.35	0.00

Table S3. Summary of model selection statistics evaluating selection on fast-start performance in *Gambusia hubbsi* (logistic regressions with survival as dependent variable). Models are ordered from best to worst. Performance abbreviations follow the main text. Bold text indicates the selected model. While two other models were not dramatically less predictive than the selected model, the added variables in these models (v_{max}, d_{net}) were never significant ($P = 0.35$, $P = 0.67$, respectively). In contrast, the two variables present in the selected model ($\bar{\omega}_{\mathrm{S1}} + a_{\mathrm{max}}$) were significant in all of these models (all one-tailed $P < 0.01$). Thus, I only present results from the model having the lowest AIC_c and highest Akaike weight.

Model	AIC_c	ΔAIC	Akaike weight
$\bar{\omega}_{\mathrm{S1}} + a_{\mathrm{max}}$	**48.78**	**0.00**	**0.40**
$\bar{\omega}_{\mathrm{S1}} + a_{\mathrm{max}} + v_{\mathrm{max}}$	50.21	1.44	0.19
$\bar{\omega}_{\mathrm{S1}} + a_{\mathrm{max}} + d_{\mathrm{net}}$	50.91	2.13	0.14
$\bar{\omega}_{\mathrm{S1}} + a_{\mathrm{max}} + v_{\mathrm{max}} + d_{\mathrm{net}}$	52.59	3.81	0.06
a_{max}	52.37	3.59	0.07
$\bar{\omega}_{\mathrm{S1}}$	53.39	4.61	0.04
$a_{\mathrm{max}} + v_{\mathrm{max}}$	53.85	5.08	0.03
$a_{\mathrm{max}} + d_{\mathrm{net}}$	54.11	5.34	0.03
$\bar{\omega}_{\mathrm{S1}} + v_{\mathrm{max}}$	55.14	6.36	0.02
$\bar{\omega}_{\mathrm{S1}} + d_{\mathrm{net}}$	55.58	6.81	0.01
$a_{\mathrm{max}} + v_{\mathrm{max}} + d_{\mathrm{net}}$	56.13	7.35	0.01
$\bar{\omega}_{\mathrm{S1}} + v_{\mathrm{max}} + d_{\mathrm{net}}$	57.17	8.40	0.01
v_{max}	59.89	11.11	0.00
d_{net}	60.51	11.74	0.00
$v_{\mathrm{max}} + d_{\mathrm{net}}$	61.25	12.47	0.00

Figure S1. Morphological variation described by the canonical variate axis from Langerhans *et al.* (2007), illustrated using thin-plate spline transformation grids relative to mean landmark positions (observed range of variation depicted). Solid lines connecting outer landmarks are drawn to aid interpretation. Fish with scores toward the left end of the axis are found in low-predation blue holes, while fish with scores toward the right end of the axis are found in high-predation blue holes. Note the large difference in size of the caudal peduncle region (highlighted). Representative live photographs of males from both predator regimes are provided beneath the grids (individuals selected near the mean body shape for low- and high-predation populations).