
Predation's Role in Life-History Evolution of a Livebearing Fish and a Test of the Trexler-DeAngelis Model of Maternal Provisioning.
Author(s): Rüdiger Riesch, Ryan A. Martin, and R. Brian Langerhans
Reviewed work(s):
Source: *The American Naturalist*, Vol. 181, No. 1 (January 2013), pp. 78-93
Published by: The University of Chicago Press for The American Society of Naturalists
Stable URL: http://www.jstor.org/stable/10.1086/668597
Accessed: 13/12/2012 07:21

VOL. 181, NO. 1 THE AMERICAN NATURALIST JANUARY 2013

Predation's Role in Life-History Evolution of a Livebearing Fish and a Test of the Trexler-DeAngelis Model of Maternal Provisioning

Rüdiger Riesch,* **Ryan A. Martin, and R. Brian Langerhans**

Department of Biology and W. M. Keck Center for Behavioral Biology, North Carolina State University, 127 David Clark Labs, Raleigh, North Carolina 27695

Submitted February 23, 2012; Accepted August 2, 2012; Electronically published November 29, 2012

Online enhancement: appendix. *Dryad data:* http://dx.doi.org/10.5061/dryad.pd383.

ABSTRACT: Populations experiencing consistent differences in predation risk and resource availability are expected to follow divergent evolutionary trajectories. For example, live-history theory makes specific predictions for how predation should drive life-history evolution, and according to the Trexler-DeAngelis model for the evolution of matrotrophy, postfertilization maternal provisioning is most likely to evolve in environments with consistent, high levels of resource availability. Using the model system of Bahamas mosquitofish (*Gambusia hubbsi*) inhabiting blue holes with and without the piscivorous bigmouth sleeper (*Gobiomorus dormitor*), we provide some of the strongest tests of these predictions to date, as resource availability does not covary with predation regime in this system, and we examine numerous (14) isolated natural populations. We found clear evidence for the expected life-history divergence between predation regimes and empirical support of the Trexler-DeAngelis model. Moreover, based on molecular and lab-rearing data, our study offers strong evidence for convergent evolution of similar life histories in similar predation regimes, largely matching previous phenotypic patterns observed in other poeciliid lineages (*Brachyrhaphis* spp., *Poecilia reticulata*), and further supports the notion that matrotrophy is most likely to evolve in stable high-resource environments.

Keywords: adaptive radiation, convergent evolution, divergent natural selection, ecological speciation, *Gambusia*, Poeciliidae.

Introduction

The ultimate goal of life-history theory is to predict how traits will evolve under particular environmental conditions. The primary assumption of this theory is that energy is limited and does not come free. Thus, an organism continually faces trade-offs concerning the allocation of limited time and resources to different organismal functions (Stearns 1992; Roff 2002). Two fundamental strategies for maximizing fitness in the face of such trade-offs exist; organisms can either invest in traits affecting age-specific mortality or in traits affecting age-specific reproductive success (Stearns 1992; Roff 2002). Two particular questions have received special attention by empiricists in recent years: (1) what is predation's role in driving life-history trait evolution in prey species (e.g., Reznick and Endler 1982; Reznick et al. 1990, 1996; Spitze 1991; Stibor 1992; Martin 1995; Johnson and Belk 2001), and (2) what is the role of environmental conditions in the evolution of maternal provisioning strategies (e.g., Trexler 1997; Thompson et al. 2002; Marsh-Matthews and Deaton 2006; Banet and Reznick 2008; Ostrovsky et al. 2009; Banet et al. 2010)? Livebearing fishes (Poeciliidae) have been at the forefront of research trying to answer both questions, and here we use field data from the model system of the Bahamas mosquitofish, *Gambusia hubbsi*, to provide some of the strongest tests of these questions to date.

First, in guppies and at least two other livebearing fishes, *Brachyrhaphis episcopi* and *Brachyrhaphis rhabdophora*, convergent life-history evolution as a response to divergent predation regimes has been documented with individuals in high-predation environments being selected for earlier age of maturity, increased fecundity, and reduced offspring size (e.g., Reznick and Endler 1982; Reznick et al. 1990, 1996; Johnson 2001; Johnson and Belk 2001; Jennions and Telford 2002; Jennions et al. 2006). However, male life histories (in particular traits other than size at maturity and investment into reproduction) have been largely neglected (Johnson and Bagley 2011; but see, e.g., Riesch et al. 2011*a*), and the question of whether both males and females will exhibit shared and/or unique responses to divergent ecological selection has so far received little attention. Among those studies that investigated sex-specific effects, most have reported a mixture of shared and unique responses of the sexes during population divergence—

---

* Corresponding author; e-mail: rwriesch.evolutionarybiology@gmail.com.

Am. Nat. 2013. Vol. 181, pp. 78–93. © 2012 by The University of Chicago. 0003-0147/2013/18101-53676$15.00. All rights reserved.
DOI: 10.1086/668597

mostly in morphological traits (e.g., Langerhans et al. 2004; Hendry et al. 2006; Langerhans and Makowicz 2009). Furthermore, in most poeciliid systems studied to date, differences in predation regime between populations are confounded with differences in resource availability (e.g., Endler 1995; Johnson 2002; Magurran 2005), making it difficult to convincingly establish that predation is the primary agent driving phenotypic shifts. Thus, more tests are needed in other species in which confounding factors are not as apparent, and *G. hubbsi* provides exactly such a system (see below).

Second, understanding the causes of variation in the way organisms provide nutrients to their embryos is a major question in life-history evolution. One variant is lecithotrophy, in which all nutrients necessary for development are provided by the yolk of the egg (yolk-feeding), while another variant is matrotrophy, which is characterized by postfertilization transfer of nutrients from a mother to her developing offspring (e.g., Wourms 1981; Marsh-Matthews 2011; Pires et al. 2011). However, it is important to note that matrotrophy and lecithotrophy only characterize endpoints of a continuum, with many intermediate strategies existing. In other words, the relative importance of nutrient transfer in overall provisioning varies extensively among matrotrophic species (Blackburn 1992, 2005), with some contributing only minor amounts of total nutrients and energy relative to yolk (incipient matrotrophy, e.g., in some viviparous lizards; Thompson et al. 2002), while others provide nearly all resources for embryonic development (substantial matrotrophy, e.g., in placental mammals; Blackburn 2005). For example, in many viviparous species nutrients for early development are provided by the yolk and are later supplemented by additional provisioning (Blackburn 1999).

To explain the evolution from lecithotrophy to matrotrophy, Trexler and DeAngelis (2003) devised two models based on the life history of the sailfin molly, *Poecilia latipinna*, and both models suggest that the evolution of substantial matrotrophy has been a stepwise process, with a resource-dependent context for the evolution of matrotrophy from a lecithotrophic ancestor. Based on the assumptions that matrotrophic females produced more, but smaller, eggs than lecithotrophic females and that postfertilization provisioning to developing embryos resulted in the same offspring quality for the two provisioning modes, the model predicted that (1) the matrotrophic strategy would be favored only in environments with sufficient resources to sustain maternal provisioning throughout gestation and (2) that matrotrophy could evolve only if females were able to abort developing embryos and recycle nutrients to the remaining embryos in the brood during times of nutritional stress. Furthermore, the models also predicted that matrotrophic females would be leaner and have shorter life spans (Trexler and DeAngelis 2003). Tests of the Trexler-DeAngelis model using experimental data have so far had mixed success; while there appears to be good evidence that resource availability does in fact influence the degree of matrotrophy under controlled laboratory conditions in both fishes with substantial matrotrophy and those with incipient matrotrophy (Marsh-Matthew and Deaton 2006; Pires et al. 2007; Pollux and Reznick 2011), evidence for the other predictions and assumptions of the Trexler-DeAngelis model has been equivocal (pro: Trexler 1997; Marsh-Matthews and Deaton 2006; contra: Marsh-Matthews and Deaton 2006; Banet and Reznick 2008; Banet et al. 2010). To date, however, no study has attempted to test the assumptions and predictions of the Trexler-DeAngelis model using data of specimens collected in natural populations. Furthermore, although predation is a prime factor in life-history evolution, the majority of existing models do not consider the role of predation (if any) in the evolution of maternal provisioning strategies (but see Trexler et al. 2011).

Bahamas mosquitofish are an ideal model system to address these important questions concerning the evolution of life-history strategies (Langerhans et al. 2007). During the past approximately 15,000 years (Fairbanks 1989), *G. hubbsi* have colonized many inland blue holes (i.e., water-filled vertical caves), which are analogous to aquatic islands in a sea of land, resulting in some of the highest F_{ST} values reported for fish populations (Schug et al. 1998; Langerhans et al. 2007). In some of these blue holes, *G. hubbsi* experience relative predator-free environments that are devoid of piscivorous fish (in fact, besides perhaps piscivorous invertebrates, no piscivores of any kind are known from these blue holes); in other blue holes, *G. hubbsi* are preyed on by the bigmouth sleeper (*Gobiomorus dormitor*; e.g., Langerhans et al. 2007; R. A. Martin and R. B. Langerhans, unpublished manuscript), and analyses of population densities and age structure suggest that all age classes suffer higher mortality (J. L. Heinen et al., unpublished manuscript). For several reasons, this system provides the perfect "'natural experiment" to test the effects of predation-mediated natural selection on life-history diversification in poeciliid fishes: blue holes represent relatively stable, constant environments (see J. L. Heinen et al., unpublished manuscript), blue holes with divergent predation regimes do not systematically differ in previously measured environmental variables (e.g., resource levels, water clarity, salinity, surface diameter; Langerhans et al. 2007; J. L. Heinen et al., unpublished manuscript), and population genetic evidence suggests that both Bahamas mosquitofish and bigmouth sleepers have been relatively isolated within many blue holes for thousands of years (Langerhans et al. 2007; R. A. Martin and R. B. Langerhans, unpublished data).

This study presents the first in-depth analysis of life-history evolution of both sexes of *G. hubbsi* as a function of divergent predation regimes between blue holes, as well as the first test of the Trexler-DeAngelis model using life-history data collected in the wild. In agreement with life-history theory (Gadgil and Bossert 1970; Law 1979; Michod 1979) and previous studies on poeciliid fishes (Reznick and Endler 1982; Reznick et al. 1990, 1996; Downhower et al. 2000; Johnson 2001; Johnson and Belk 2001), we predicted to find *G. hubbsi* from high-predation localities to produce more but smaller offspring, invest more into reproduction, and to be smaller than their counterparts from low-predation environments. Based on previous work in the genus *Gambusia* that found fishes from high-predation sites to have larger midbody/caudal regions and possess greater fast-start performance (Langerhans et al. 2004, 2007; Langerhans 2009*a*, 2009*b*; Langerhans and Makowicz 2009), we also predicted fish from high-predation sites to have a higher size-corrected lean weight, reflecting a greater investment into muscle mass. Furthermore, based on the Trexler-DeAngelis model, *G. hubbsi* from blue holes with high resource availability should show higher levels of postfertilization maternal provisioning, which in turn should lead to less body fat in females, smaller oocytes at fertilization, and higher fecundity. Also, if maternal provisioning in fact differed between environments, we would predict to find a higher incidence of embryo abortion in low-resource environments. For all life-history traits with existing relevant data, we further tested whether population differences have a genetic basis.

Methods

Study Populations

We collected *Gambusia hubbsi*, using dip nets and minnow traps from 14 blue holes (seven with predators, seven without; fig. A1, available online) on Andros Island, Bahamas, during the first 2 weeks of May 2011. Immediately after collection we euthanized the fish in an aqueous solution of tricane methanesulfonate (MS 222) and preserved them in 95% ethanol.

Simultaneous with fish collections, we measured direct estimates of resource availability in each blue hole. Because Bahamas mosquitofish exhibit a broad diet (Gluckman and Hartney 2000; R. A. Martin and R. B. Langerhans, unpublished data), it is not clear how to best estimate resource availability for these fish. Therefore, we measured a range of variables designed to capture relevant aspects of overall productivity of blue holes (Grether and Kolluru 2011). To estimate total algal and cyanobacteria biomass, we measured the photosynthetic pigments chlorophyll *a* and phycocyanin, respectively, in a water sample using a fluorometer (AquaFluor, Turner Designs, Sunnyvale, CA). Zooplankton and phytoplankton densities were estimated using a 60-m tow of a zooplankton net (20-cm diameter, 153-μm mesh) at 0.5-m depth. All plankton were counted within a 2.5-mL subsample of each plankton collection using a stereo microscope. Phytoplankton were divided into two size classes, small (<0.05 mm^2) and large (≥ 0.05 mm^2).

In a preliminary analysis, we wanted to screen whether our measures of resource availability were correlated with predation regime. We first conducted a principal components analysis (on the correlation matrix) to reduce the number of factors derived from our five measures of resource availability (chlorophyll *a*, phycocyanin, zooplankton density, small phytoplankton density, and large phytoplankton density). Principal component (PC) axes with an eigenvalue over 1 were retained as resource availability variables (2 axes retained accounting for 64.5% of the total variation; PC1 primarily corresponds to chlorophyll *a* and phycocyanin, and PC2 to small phytoplankton, large phytoplankton, and zooplankton; table A1, available online). We then conducted a MANOVA with "resource PC1" and "resource PC2" as the dependent variables and "predation regime" as the independent variable. As expected, variation in resource availability between populations was independent of predation regime ($F_{2,11} = 0.317$, $P = .735$), supporting our assumption that there is little to no covariance between resource availability and predation regime in *G. hubbsi* from Bahamas blue holes.

Life-History Measurements

Following the protocol of Reznick and Endler (1982), all preserved fish were weighed and measured for standard length. In the case of females, the reproductive tissue and, if present, all developing offspring were removed. Offspring were counted and their stage of development determined (Reznick 1981; Riesch et al. 2011*b*), which included classifying those embryos as aborted that showed definitive characteristics of advanced decomposition (see the appendix, available online, for details). Somatic tissues, reproductive tissues and embryos were then dried for 24 h at 55°C and weighed again. To assess female and embryo condition, somatic tissues and embryos were rinsed six times for at least 6 h in petroleum ether to extract soluble nonstructural fats (Heulett et al. 1995; Marsh-Matthews et al. 2005) and were then redried and reweighed. Furthermore, we calculated reproductive allocation (RA) by dividing offspring weight by the sum of offspring weight plus somatic dry weight (Reznick and Endler 1982).

Males were classified as mature based on the morphology of their modified anal fin (i.e., gonopodium). The anal fin undergoes a complex metamorphosis as fish attain maturity, and the endpoint of this metamorphosis provides a reliable index of sexual maturity, allowing the definition of complete maturation based on external cues alone (e.g., Kallman and Schreibman 1973; Schreibman and Kallman 1977; Reznick et al. 1993). Even though there are slight differences among species, the general metamorphosis is similar to that described by Turner (1941) for *Gambusia affinis*. Finally, we calculated the gonadosomatic index (GSI) by dividing a male's reproductive tissue dry weight by the sum of reproductive tissue dry weight and somatic dry weight (Riesch et al. 2011*a*).

We thus collected the following male and female life-history traits: standard length (SL [mm]), dry weight (g), lean weight (g), and fat content (%). Furthermore, for males we also collected GSI (%), and for females fecundity (no. developing offspring), offspring dry weight (mg), offspring fat content (%), and RA (%).

We then $\log_{10}$ transformed (male/female SL, male/female lean weight, and embryo dry/lean weight), square root transformed (fecundity), or arcsine(square root) transformed (male/female fat content, embryo fat content, female RA, and male GSI) all life-history variables to meet assumptions of statistical analyses (i.e., these transformations greatly facilitated normality of model residuals). To remove size/allometry effects on life-history traits other than SL, we regressed these variables against SL (and for females also embryonic stage of development) separately for each sex, confirmed homogeneity of slopes among predation regimes ($P > .31$ in all cases), and then used residuals from these models in all subsequent analyses.

Population Comparison of Female and Male Life Histories

We conducted mixed-model multivariate analysis of variance for our primary tests of phenotypic differences between predation regimes, and analyses were conducted separately for each sex. Phenotypic traits described above served as dependent variables. We tested for effects due to "predation regime" and "population nested within predation regime" (random effect; hereafter population[predation regime]). Statistical significance was determined as follows: F approximation from Wilks's Λ for population nested within predation regime, and F-test using restricted maximum likelihood and the Kenward-Roger degrees of freedom adjustment (Kenward and Roger 1997) for predation regime to appropriately test this fixed effect while treating population as a random term (i.e., effectively treating population as the unit of replication). The latter significance test was conducted using the MIXED procedure in SAS (SAS Institute, Cary, NC; sample code in the appendix), while all other tests were conducted in JMP (SAS). We evaluated the relative importance of model terms using the effect size measurement of Wilks's partial η^2 (measure of partial variance explained by a particular term; multivariate approximation of $SS_{effect}\backslash[SS_{effect} + SS_{error}]$; see appendix of Langerhans and DeWitt 2004). To provide an intuitive metric for understanding the magnitude of trait differences between predation regimes, we also present standardized effect sizes (Cohen's d, describing the difference between means in standard deviation units; Cohen 1988).

Shared and Unique Responses between the Sexes

For the life-history traits common to both sexes (those not involving developing offspring), we directly examined the magnitude and nature of shared and unique responses to predation regime among the sexes using a mixed-model multivariate analysis. For this analysis, we analyzed the following male and female life histories: male/female SL, male/female lean weight, male/female fat content, and male/female investment into reproduction (RA for females and GSI for males). All variables were again $\log_{10}$- or arcsine(square root)-transformed as described above and the confounding effects of SL were removed by using the residuals from a preparatory multivariate regression of the four traits on SL. We performed a mixed-model multivariate analysis of variance as above, but this time our model included "predation regime," "sex," "population(predation regime)," and the interaction of "sex-by-predation regime" as independent variables.

Genetic Basis of Population Differences in Female Life-History Traits

Downhower et al. (2000) presented two relevant data sets for testing whether there is a genetic basis to differences in female life-history traits between blue hole populations of Bahamas mosquitofish. We extracted and analyzed data from that publication to test for a genetic basis for between-population variation in fecundity, embryo size, and reproductive investment.

First, fish from three populations (one low- and two high-predation sites) were collected in the wild, offspring were acquired in the lab, and F1 offspring were raised under common ad lib. food conditions. Fecundity was measured for wild-caught fish in the field, parental stock raised under lab conditions, and F1 offspring born and raised in the lab. We obtained fecundity values for these fish from table 5 in Downhower et al. (2000), standardized values within generation to remove generational effects (treating generation as a blocking factor; fish from all populations produced higher fecundity in the lab), and tested for mean differences between populations using model II ANOVA.

Second, fish from four blue hole populations (two low-

---

[1] Code that appears in the *American Naturalist* is provided as a convenience to the readers. It has not necessarily been tested as part of the peer review.

predation, one high-predation, and one medium-predation site; the latter containing only *Lutjanus griseus*, a generalist predator that typically includes some fish in its diet) were introduced into well fields on Andros Island that were previously devoid of fish. It was assumed that these fish subsequently experienced similar conditions in these well fields. Fish were collected approximately 1–2 generations later (9–14 months) and again approximately 2–4 generations later (22–26 months), and fecundity, egg diameter, and reproductive investment (log (egg volume × average litter)/total length) were measured in their original population and during each sampling period. We obtained fecundity, egg diameter, and reproductive investment measures for these fish from table 4 in Downhower et al. (2000) and performed analysis as described above. However, since egg diameter exhibited nearly no variance after 26 months (partially due to values having only a single decimal place in the table, with original differences only being 0.1–0.2 mm), we analyzed egg diameter across only 1–3 generations (Downhower et al. 2000).

Genetic Independence of G. hubbsi *Populations*

To evaluate population genetic structure and test whether populations within predation regimes may be more closely related to one another than to populations in the alternative predation regime, we examined molecular genetic data. We sequenced a 886-bp fragment of the NADH subunit 2 (ND2) mitochondrial gene following Langerhans et al. (2007) for approximately five specimens for each of the 14 populations examined ($N = 72$, 5.1 ± 0.1 per population), and conducted an analysis of molecular variance (AMOVA) testing for three sources of molecular genetic variance: between predation regimes, among populations within predation regimes, and within populations.

Maternal Provisioning

We first tested for differences in maternal provisioning between predation regimes by means of a mixed-model ANCOVA, in which embryo dry weight was the dependent variable, "predation regime" the independent variable, "population(predation regime)" a random effect, and embryonic stage of development the covariate. We also included the interaction of "predation regime-by-embryonic stage of development" to evaluate whether the degree of weight loss differed between predation regimes.

Second, to evaluate the mode of maternal provisioning, we calculated the matrotrophy index (MI) using the slopes and intercepts from the regression analysis described below. The MI equals the estimated dry mass of the embryo at birth divided by the estimated dry mass of the ovum at fertilization (e.g., Reznick et al. 2002; Riesch et al. 2010, 2011*b*). If the eggs were fully provisioned by yolk before fertilization (lecithotrophy), then we would expect the embryos to lose 25%–40% of their dry mass during development (MI between 0.60 and 0.75; Scrimshaw 1945; Wourms 1981). On the other hand, in the case of continuous maternal provisioning after fertilization (matrotrophy), one would expect the embryos to lose less weight (MI between 0.75 and 1.00) or to even gain weight during development ($\mathrm{MI} > 1.00$; e.g., Reznick et al. 2002). Thus, maternal provisioning was evaluated by analyzing the relationship between log-transformed embryonic dry mass and stage of development by means of linear regression analysis (Reznick et al. 2002; Riesch et al. 2010, 2011*b*). However, we excluded two populations (Rivean's and Runway blue holes) from all analyses of maternal provisioning due to low sample sizes ($N \leq 7$).

We then tested each population separately for significant divergence from an MI of 0.7 (a cutoff, below which represents lecithotrophy and above which represents at least some level of matrotrophy) by testing each population against that population's hypothetical slope for an MI of 0.7 in one-sample *t*-tests (following Reznick et al. 2002).

Testing the Trexler-DeAngelis Model of Maternal Provisioning

To test whether fecundity, oocyte mass at fertilization (using the *Y*-axis intercept from the regression analysis described above), and female fat content are a function of the rate of maternal provisioning (i.e., the assumptions of the Trexler-DeAngelis model), we ran three separate general linear models to investigate the influence of predation regime, resource PC1, resource PC2, and MI on these three variables (the former three terms were included in the models so that the test of MI controlled for possible effects of resource availability and predation risk).

To test for the influence of resource availability on the rate of postfertilization maternal provisioning (prediction 1), we ran a general linear model in which MI was the dependent variable and the independent variables were "predation regime," "resource PC1," and "resource PC2" (see above) as well as all possible two-way interactions; because none of the interactions were statistically significant ($P > .3$ in all cases), we subsequently removed them from the final model.

Finally, to test if females from low-resource environments exhibit a higher incidence of abortion than females from resource-abundant habitats (prediction 2), we calculated the proportional "abortion incidence" (AI) for each female by dividing the number of aborted embryos by the sum of the number of aborted embryos and fecundity. We then ran a general linear model, in which arcsine(square root)-transformed population means for AI was the dependent variable and the independent variables were "predation regime," "resource PC1," and "resource PC2" (see above) as well as all possible two-way interactions; nonsignificant interactions were removed from the final model in a stepwise process ($P > .5$ in both cases).

Results

Population Comparison

Descriptive statistics for female life history traits are summarized in table 1. We did not find any evidence for superfetation in *Gambusia hubbsi* as only 2% (4 out of 198) of pregnant females had embryos spanning more than one developmental stage and in only one of those four cases were the two subclutches of nonconsecutive developmental stages. In the mixed-model nested MANOVA for female *G. hubbsi* both "predation regime" and "population(predation regime)" had a significant influence on life-history traits (tables 1, 2A); however, according to our measure of effect size (η_p^2), predation regime had by far the strongest influence on female life histories (table 2A). Evaluation of the canonical axis derived from the predation regime term of the MANOVA revealed that this is mainly due to differences in female lean weight, fecundity, embryo lean weight, and embryo fat content (fig. 1, table A2, available online). Evaluation of the descriptive statistics revealed that females in high-predation blue holes were characterized by higher lean weight, higher fecundity, lower embryo fat content, and lower embryo lean weight compared to females from low-predation blue holes (table 1).

Descriptive statistics for male life histories are summarized in table 3. Both factors "predation regime" and "population(predation regime)" had a significant influence on male life-history traits in the mixed-model nested MANOVA (tables 2B, 3) and predation regime, albeit weaker compared to females (table 2A) again had the strongest influence on life histories (table 2B). Evaluation of the canonical axis derived from the predation regime term of the MANOVA revealed that this is mainly due to differences in male lean weight and GSI (fig. 1, table A2). Evaluation of the descriptive statistics revealed that males in high-predation blue holes were characterized by increased lean weight and GSI compared to males from low-predation blue holes (table 3).

Shared and Unique Responses between the Sexes

As expected, "sex," "predation regime," and "population(predation regime)," as well as the interaction of "sex-by-predation regime" had significant effects on life histories in the mixed-model nested MANOVA (table 2C). Clearly, the strongest differentiation was observed between males and females (sex: $\eta_p^2 = 91.9\%$; table 2C); however, "predation regime" ($\eta_p^2 = 37.5\%$) and "population(predation regime)" ($\eta_p^2 = 20.3\%$) also had strong effects on life histories, while the effect of "sex-by-predation regime" was fairly weak ($\eta_p^2 = 4.8\%$; table 2C). We inspected the canonical axes derived from these terms in the model to interpret the causes of each effect (table A3, available online). This revealed that the sex effect is mainly due to sex-specific differences in SL and reproductive investment (GSI vs. RA) as males are smaller than females and invest less into reproduction (GSI < RA; tables 1, 3). The shared response (effect of predation regime) is mostly a result of both sexes increasing in lean weight in high-predation blue holes (tables 1, 3), and the interaction of "sex-by-predation regime" is driven by differences in fat content and reproductive investment; while females in low-predation blue holes are characterized by higher fat content and RA compared to females in high-predation blue holes, this trend is opposite in males (tables 1, 3).

Genetic Basis of Population Differences in Female Life-History Traits

Using data from Downhower et al. (2000), we first found that for the laboratory experiment, populations significantly differed from one another in fecundity ($P = .0091$), indicating that populations largely maintained fecundity differences observed in the field after rearing under common lab conditions (intraclass correlation coefficient: $r = 0.78$). Second, for the well-field experiment, we found that populations significantly differed from one another in fecundity ($P = .0002$), egg diameter ($P = .0357$), and reproductive investment ($P = .0283$), revealing that ancestors and descendants within populations exhibited life histories more similar to one another than to fish from other populations (intraclass correlation coefficient: fecundity, $r = 0.88$; egg diameter, $r = 0.78$; reproductive investment, $r = 0.58$).

Genetic Independence of G. hubbsi *Populations*

We found strong population genetic structure, consistent with colonization of blue holes thousands of years ago with minimal gene flow ever since. Moreover, we found strong evidence that genetic relatedness is not associated with predation regime (tables A4, A5; fig. A4, available online).

Maternal Provisioning

The extent of maternal provisioning as measured by the matrotrophy index varied widely among *G. hubbsi* pop-

Table 1: Descriptive statistics for female *Gambusia hubbsi* life histories

Predation regime, population	N	SL (mm)	Female fat content[a] (%)	Female lean weight[b] (g)	Fecundity[b] (no. offspring)	RA (%)	Embryo lean weight[a] (mg)	Estimated embryo dry weight at birth[c] (mg)	Embryo fat content[a] (%)
Low predation:									
East Twin	11	30.35 ± 3.94	.74 ± 1.99	.09 ± .01	2.65 ± 1.65	10.34 ± 3.16	3.51 ± .16	3.91	2.71 ± 4.64
Gollum's	12	29.91 ± 3.42	1.15 ± 2.08	.10 ± .01	2.90 ± 1.64	13.64 ± 4.16	4.20 ± .15	3.97	11.89 ± 4.85
Hubcap	19	24.64 ± 1.62	2.76 ± 2.00	.11 ± .01	3.97 ± 1.73	11.54 ± 3.29	3.80 ± .12	4.06	7.80 ± 4.79
Ken's	28	30.09 ± 2.83	2.95 ± 2.12	.09 ± .01	2.63 ± 1.66	13.25 ± 4.17	4.55 ± .10	4.50	10.89 ± 4.76
Pigskin	21	27.94 ± 4.33	3.13 ± 1.83	.11 ± .01	3.75 ± 1.63	12.64 ± 2.18	4.12 ± .11	4.13	10.27 ± 4.58
Rainbow	25	26.29 ± 2.49	1.86 ± 1.80	.09 ± .01	3.73 ± 1.67	14.33 ± 3.55	4.02 ± .10	4.01	7.43 ± 5.00
Voy's	10	28.40 ± 3.67	2.88 ± 1.90	.10 ± .01	4.84 ± 1.63	14.72 ± 4.60	3.99 ± .16	3.25	6.75 ± 4.74
Total/avg	126	28.03 ± 3.70	2.37 ± 2.24	.10 ± .01	3.43 ± 1.96	13.00 ± 3.74	4.09 ± .06	3.98	8.67 ± 5.61
High predation:									
Cousteau's	9	35.29 ± 2.82	2.81 ± 2.10	.12 ± .01	9.10 ± 1.80	15.14 ± 2.32	2.82 ± .17	2.70	7.93 ± 4.80
Hard Mile	9	28.06 ± 3.89	2.50 ± 2.10	.11 ± .01	3.46 ± 1.63	10.49 ± 1.56	3.40 ± .17	3.28	3.05 ± 4.80
Rivean's	5	29.84 ± 3.44	1.11 ± 2.01	.11 ± .01	6.79 ± 1.63	12.99 ± 1.98	2.35 ± .23	...	3.32 ± 4.92
Runway	7	25.04 ± 2.07	2.05 ± 2.12	.12 ± .01	9.28 ± 1.66	12.43 ± 2.98	1.32 ± .20	...	1.27 ± 4.76
Shawn's	22	26.93 ± 4.52	1.59 ± 1.88	.13 ± .01	7.99 ± 1.64	10.19 ± 1.65	1.51 ± .11	1.52	1.90 ± 4.69
Stalactite	10	30.36 ± 1.92	1.67 ± 1.90	.10 ± .01	6.21 ± 1.64	15.16 ± 2.54	3.10 ± .16	2.84	2.91 ± 4.74
West Twin	10	27.95 ± 2.17	.84 ± 1.90	.11 ± .01	7.18 ± 1.63	13.02 ± 2.58	2.17 ± .16	1.95	2.27 ± 4.74
Total/avg	72	28.75 ± 4.37	1.77 ± 1.70	.11 ± .02	7.26 ± 1.97	12.34 ± 2.90	2.27 ± .09	2.46	3.06 ± 5.09
Cohen's d		.18	.30	.63	1.95	.20	23.80	...	1.05

Note: Presented are means ± SD for life-history traits of female *Gambusia hubbsi* from seven high-predation and seven low-predation blue holes collected on Andros Island, Bahamas. RA = reproductive allocation; SL = standard length. Cohen's d is the standardized effect size for the predator regime term.

[a] Estimated marginal means from a MANCOVA with stage of development as a covariate.

[b] Estimated marginal means from a MANCOVA with SL as a covariate.

[c] Estimated using the intercept and slope from a regression of log-transformed embryo dry weight against stage of development.

Table 2: Multivariate results for life-history differentiation

Factor	F	df	P	Partial variance explained (%)
A. Females:				
Predation regime	123.08	6, 575	<.0001	80.5
Population (predation regime)	7.66	84, 1,098	<.0001	33.2
B. Males:				
Predation regime	13.55	3, 253	<.0001	49.1
Population (predation regime)	4.44	48, 476	<.0001	31.3
C. Both sexes combined:				
Sex	909.59	4, 319	<.0001	91.9
Predation regime	22.29	3, 678	<.0001	37.5
Sex × predation regime	4.01	4, 319	.0035	4.8
Population (predation regime)	6.80	48, 1,231	<.0001	20.3

Note: Results from the mixed-model nested MANOVAs on differentiation of life-history traits of *Gambusia hubbsi* from seven high- and seven low-predation blue holes collected on Andros Island, Bahamas (treating population as a random effect). A, Female-only analysis; B, male-only analysis; and C, analysis on shared and unique responses between the sexes. The F-statistics and partial variance (partial η^2) were calculated as described in the text.

ulations (fig. 2), and the MI values indicate that some populations have little or no maternal provisioning ($0.7 < \mathrm{MI} < 0.75$), while others have moderate amounts of maternal provisioning ($0.8 < \mathrm{MI} < 1.1$). Nonetheless, while "predation regime," "population(predation regime)," and "developmental stage" significantly affected embryo weight, predation regime had no effect on the degree of weight loss across developmental stages (ANCOVA, predation regime: $F_{1,14} = 9.976$, $P = .007$; population(predation regime): $F_{10,175} = 32.427$, $P < .0001$; developmental stage: $F_{1,175} = 14.468$, $P < .0001$; predation regime-by-developmental stage: $F_{1,175} = 0.854$, $P = .357$). However, when statistically comparing whether or not there was maternal provisioning after fertilization, several populations had an MI that was significantly greater than 0.7 (Ken's: $t_{27} = 2.2$, $P = .037$; Hard Mile: $t_8 = 2.4$, $P = .043$; Hubcap: $t_{17} = 3.6$, $P = .002$; Rainbow: $t_{24} = 2.6$, $P = .016$).

Testing the Trexler-DeAngelis Model of Maternal Provisioning

Fecundity and estimated oocyte mass at fertilization clearly differed between predation regimes as expected but were otherwise not strongly influenced by MI or resource availability, with the exception of a suggestive trend for decreasing oocyte size with increasing MI (fecundity: predation regime, $F_{1,7} = 6.894$, $P = .034$; MI, $F_{1,7} = 0.730$, $P = .421$; resource PC1, $F_{1,7} = 1.837$, $P = .217$; resource PC2, $F_{1,7} = 0.009$, $P = .924$; oocytes mass: predation regime, $F_{1,7} = 16.142$, $P = .005$; MI, $F_{1,7} = 3.825$, $P = .091$, fig. 3*A*; resource PC1, $F_{1,7} = 1.262$, $P = .298$; resource PC2, $F_{1,7} = 0.086$, $P = .778$). Similarly, female fat content was not affected by any of the predictor variables, with the exception of a suggestive trend for increased female fat in blue holes with more algal biomass (predation regime, $F_{1,7} = 2.476$, $P = .160$; MI, $F_{1,7} = 1.863$, $P = .215$, fig. 3*B*; resource PC1, $F_{1,7} = 4.149$, $P = .081$; resource PC2, $F_{1,7} = 0.338$, $P = .579$).

Resource PC 2 had a significant influence on between-population variation in MI ($F_{1,8} = 7.339$, $P = .027$), while effects of predation regime ($F_{1,8} = 1.716$, $P = .23$) and resource PC 1 ($F_{1,8} = 1.345$, $P = .28$) were not significant. The amount of maternal provisioning increased with increased availability of phytoplankton and zooplankton (fig. 3*C*). Results were similar when weighting MI values by population sample sizes (PC2, $P = .029$; PC1, $P = .30$; predation regime, $P = .40$), indicating that sampling error within populations having different sample sizes does not explain observed findings.

A small percentage of females from almost all populations examined (11.3% overall) had one or more aborted embryos (1.45 ± 1.53, mean ± SD) among the developing clutch (the exception being females from Rivean's, Runway, and Stalactite). Only the interaction between "predation regime-by-resource PC 1" had a significant influence on between-population variation in abortions ($F_{1,9} = 5.163$, $P = .049$), while "predation regime" ($F_{1,9} = 0.922$, $P = .362$) and "resource PC2" ($F_{1,9} = 0.001$, $P = .972$) were not significant, and "resource PC 1" ($F_{1,9} = 4.274$, $P = .081$) was marginally nonsignificant. Visual inspection of the relationship between resource PC1 on AI for each predation regime indicated that the interaction effect reflected that AI was positively correlated with resource availability in low-predation blue holes but negatively correlated with resource availability in high-pre-

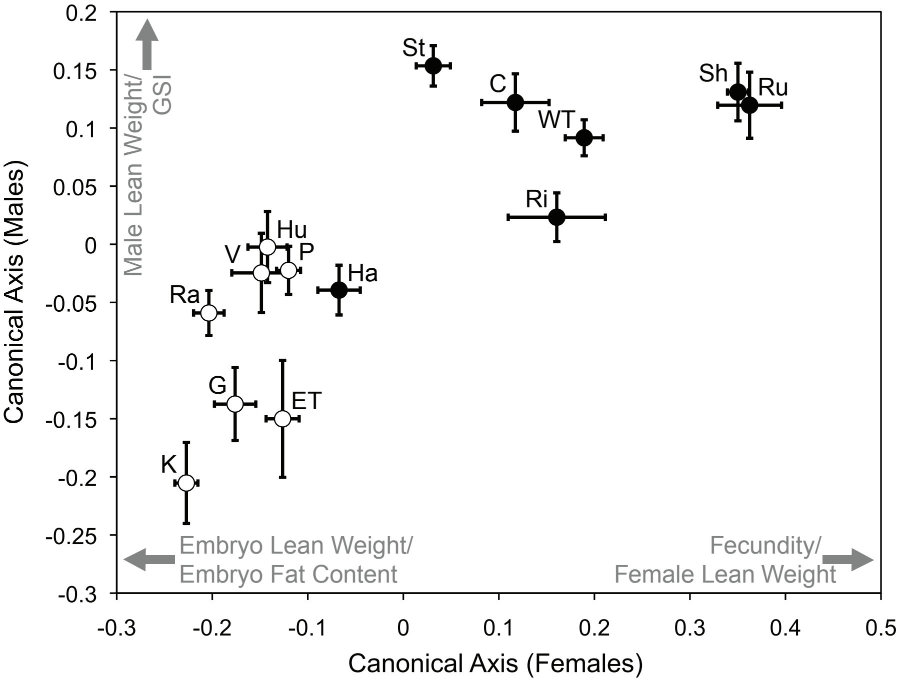

Figure 1: Population variation for *Gambusia hubbsi* of both sexes in multivariate axes (canonical variate from predation regime term) of life-history divergence between predation regimes. Means and standard errors depicted. Open circles: low-predation blue holes; filled circles: high-predation blue holes. Populations: Cousteau's (C), East Twin (ET), Gollum's (G), Hard Mile (Ha), Hubcap (Hu), Ken's (K), Pigskin (P), Rainbow (Ra), Rivean's (Ri), Runway (Ru), Shawn's (Sh), Stalactite (St), Voy's (V), and West Twin (WT).

dation blue holes (fig. A5, available online). However, this interaction was mainly driven by one blue hole, Gollum's, which had extremely high values for both phycocyanin and chlorophyll *a* (fig. 3*B*); if Gollum's was excluded from this analysis, the interaction term becomes highly nonsignificant ($P = .695$) and "resource PC1" becomes significant ($P = .03$), indicating that, generally, AI increased with decreasing resource availability in all habitats irrespective of predation regime ($R^2 = 0.458$; fig. 3*D*).

Discussion

Predation and Convergent Population Divergence in G. hubbsi

We found strong evidence for all three requirements of convergent evolution (Endler 1986) of life-history strategies in *G. hubbsi* as a response to divergent predation regimes: (1) Bahamas mosquitofish from different predation regimes diverged in predictable fashion in their life histories (i.e., phenotype-environment association), (2) at least some of this life-history divergence is heritable (i.e., genetic basis of convergent traits), and (3) genetic relatedness is not related to predation regime (i.e., convergent traits do not reflect common ancestry). These lines of evidence suggest that *G. hubbsi* populations have repeatedly evolved similar life histories in similar predation environments, analogous to previous findings for body shape evolution in this system (Langerhans et al. 2007). Our analysis also reaffirmed that most blue holes were likely colonized thousands of years ago and exhibit minimal gene flow with outside populations.

The divergent life histories documented in *G. hubbsi* are largely in agreement with predictions of life-history theory (Gadgil and Bossert 1970; Law 1979; Michod 1979) and the predictions derived from our knowledge on morphological divergence between predation regimes in this and other *Gambusia* species (Langerhans et al. 2004, 2007; Langerhans 2009*a*, 2009*b*; Langerhans and Makowicz 2009). In high-predation environments, *G. hubbsi* females produce more but smaller offspring, and both sexes are characterized by a higher relative lean weight and show a tendency for reduced body size. These patterns of life-history divergence in Bahamas mosquitofish are highly similar to those found in guppies (e.g., Reznick and Endler 1982; Reznick et al. 1993, 1996*b*), *Brachyrhaphis rhabdophora* (e.g., Johnson 2001; Johnson and Belk 2001), and

Table 3: Descriptive statistics for male *Gambusia hubbsi* life histories

Predation regime, population	*N*	SL (mm)	Male fat content[a] (%)	Male lean weight[a] (g)	GSI[a] (%)
Low predation:					
East Twin	10	22.72 ± 2.32	2.05 ± 2.85	.04 ± .01	.96 ± .32
Gollum's	10	24.50 ± 2.19	3.55 ± 2.85	.04 ± .01	1.13 ± .32
Hubcap	10	21.51 ± 2.49	2.21 ± 2.85	.05 ± .01	1.42 ± .32
Ken's	10	24.62 ± 2.34	.49 ± 2.85	.04 ± .01	1.11 ± .32
Pigskin	10	22.08 ± 3.43	1.88 ± 2.85	.05 ± .01	1.09 ± .32
Rainbow	10	23.37 ± 2.21	3.23 ± 2.85	.04 ± .01	1.57 ± .32
Voy's	10	20.68 ± 2.29	1.69 ± 2.85	.05 ± .01	1.00 ± .32
Total/avg	70	22.78 ± 2.76	2.17 ± 1.26	.05 ± .00	1.18 ± .32
High predation:					
Cousteau's	10	24.70 ± 1.94	5.19 ± 2.85	.05 ± .01	1.84 ± .32
Hard Mile	10	22.18 ± 1.45	3.56 ± 2.85	.04 ± .01	1.35 ± .32
Rivean's	10	23.97 ± 2.85	3.39 ± 2.85	.05 ± .01	1.22 ± .32
Runway	10	22.65 ± 2.19	.56 ± 2.85	.05 ± .01	1.50 ± .32
Shawn's	10	20.50 ± 2.91	2.76 ± 2.85	.05 ± .01	1.44 ± .32
Stalactite	10	22.80 ± 2.30	6.26 ± 2.85	.05 ± .01	1.76 ± .32
West Twin	10	23.61 ± 1.75	2.54 ± 2.85	.05 ± .01	1.42 ± .32
Total/avg	70	22.92 ± 2.51	3.46 ± 1.26	.05 ± .00	1.50 ± .32
Cohen's *d*		.05	1.02	0.88	1.00

Note: Presented are means ± SD for life-history traits of male *Gambusia hubbsi* from seven high-predation and seven low-predation blue holes collected on Andros Island, Bahamas. GSI = gonadosomatic index; SL = standard length. Cohen's *d* is the standardized effect size for the predator regime term.

[a] Estimated marginal means from a MANCOVA with SL as a covariate.

Brachyrhaphis episcopi (e.g., Jennions and Telford 2002) despite the fact that all three genera represent distantly related lineages within the family Poeciliidae (Hrbek et al. 2007), experience different sets of predators, inhabit different types of aquatic habitats (i.e., streams and blue holes), and live in different parts of the world. Thus, *B. rhabdophora*, *B. episcopi*, *G. hubbsi*, and *Poecilia reticulata* represent a very convincing case of both intraspecies and interspecies convergence in response to similar selective regimes. Moreover, we were able to document convergence in at least one trait between the sexes in *G. hubbsi*, and life-history divergence between predation regimes is clearly not the result of environmental correlates of predator presence in Bahamas mosquitofish (for tests of other abiotic variables between predation regimes, see Langerhans et al. 2007; J. L. Heinen et al. unpublished manuscript).

Nonetheless, not all life-history traits met our a priori predictions. For example, while males from high-predation environments did invest more into reproduction than their low-predation counterparts, we did not find this pattern in females; in fact, the trend in females was opposite in direction—a pattern more similar to *B. rhabdophora* than *P. reticulata* or *B. episcopi* (e.g., Reznick and Endler 1982; Johnson and Belk 2001; Jennions and Telford 2002). This unpredictable component of life-history evolution could reflect a trade-off in high-predation environments that *G. hubbsi* and *B. rhabdophora* females resolve differently than guppies and *B. episcopi*. Females of species with internal fertilization that carry large clutches may be at a disadvantage when escaping from predators, because high reproductive investment can impair swimming ability and fast-start responses (e.g., Olsson et al. 2000; Plaut 2002; Ghalambor et al. 2004; Belk and Tuckfield 2010), traits essential for survival in high-predation environments (Walker et al. 2005; Langerhans 2009*a*, 2009*b*). Previous work has demonstrated that *G. hubbsi* females exhibit increased fast-start escape responses in high-predation blue holes (Langerhans 2009*a*). Thus, for *G. hubbsi* (and potentially for *B. rhabdophora*) females, the fitness reduction experienced as reduced survival might outweigh the fitness increase associated with increased reproductive investment, leading to similar or even reduced reproductive allocation in high-predation populations (implying that this trade-off may be weaker or resolved differently in guppies and *B. episcopi*).

It is important to note in this context that we do not yet fully understand the exact predation pressures imposed on *G. hubbsi* by bigmouth sleepers in all of these blue holes, so some of the observed variation could potentially be due to differences in bigmouth sleeper densities or predation pressures among high-predation blue holes. For example, it is possible that bigmouth sleepers are more

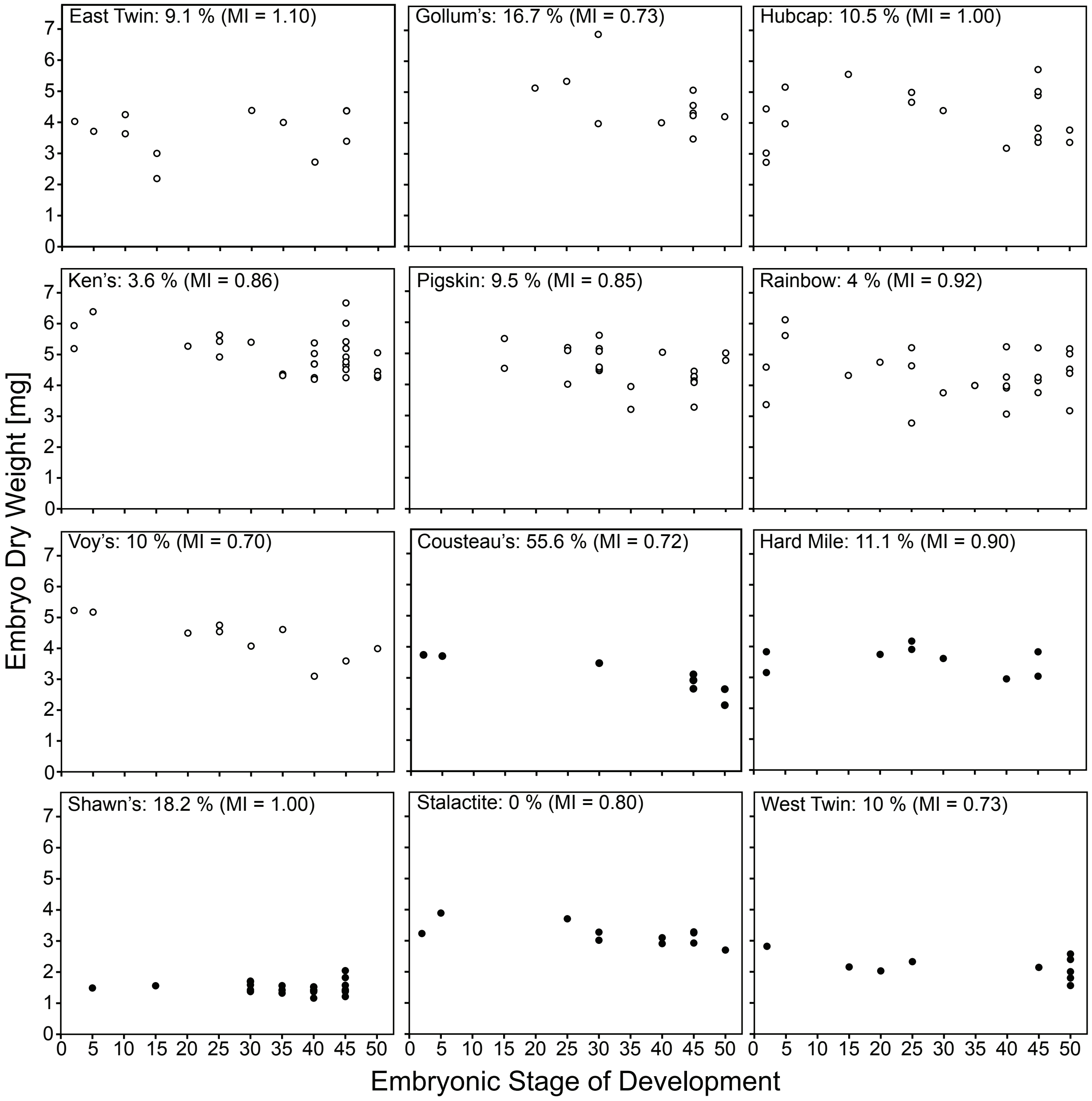

Figure 2: Scatterplots of mean embryo dry mass versus stage of development of 12 populations of *Gambusia hubbsi* from Andros Island on the Bahamas. Stages are determined on a progressive scale with the earliest (5) being the neurula stage and the oldest (50) being equivalent to embryos that are ready to be born (after Reznick 1981; Riesch et al. 2011*b*). Percentages provide the proportion of pregnant females with aborted embryos, and the matrotrophy index (MI) is the estimated dry mass at birth divided by dry mass at fertilization. Open circles: low-predation blue holes; filled circles: high-predation blue holes.

size-selective in certain populations than in others; the only constant difference between high- and low-predation blue holes is likely to be overall mortality of Bahamas mosquitofish. Traditional models of life-history theory specifically focused on size-specific mortality rates (e.g., Gadgil and Bossert 1970; Law 1979; Michod 1979), and most of our results are actually congruent with these predictions; however, recent years have seen the emergence of both theoretical (e.g., Kozlowski and Uchmanski 1987; Abrams and Rowe 1996) and empirical studies (e.g., Mattingly and Butler 1994; Reznick et al. 1996*a*; Bronikowski et al. 2002) that predict/suggest even uniform mortality

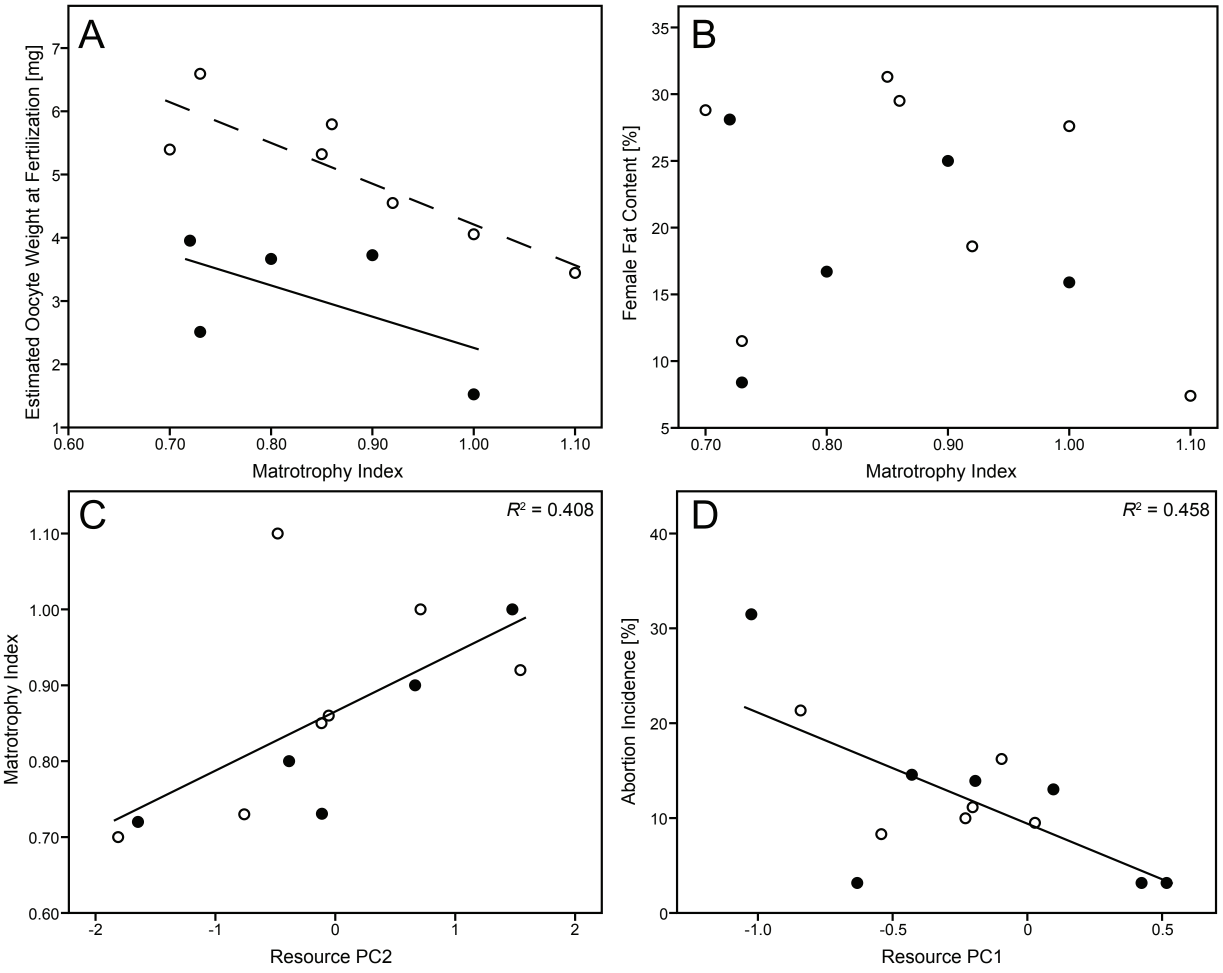

Figure 3: *A*, Regression of estimated dry weight of oocytes at fertilization versus matrotrophy index. *B*, Scatterplot of female fat content versus matrotrophy index. *C*, Regression of matrotrophy index versus resource principal component 2. *D*, Regression of abortion incidence versus resource principal component 1, after excluding the outlier population (see main text). Principal components were derived from a principal component analysis on five measures of resource availability. Open circles: low-predation blue holes; filled circles: high-predation blue holes.

rates can have measurable evolutionary impacts on life histories. Potentially, some of the differences we report on here could reflect indirect results of predation pressures on population densities (e.g., Abrams and Rowe 1996; Reznick et al. 1996*a*; Bronikowski et al. 2002), as population densities are generally lower in high-predation blue holes (J. L. Heinen et al., unpublished manuscript; R. Riesch, R. A. Martin, and R. B. Langerhans, unpublished manuscript). Future research in this system will benefit from investigating the nature of predation pressures per se (e.g., mortality rates among age/sex classes of *G. hubbsi*).

Also, males and females exhibited sex-specific divergence in body condition between predation regimes: males had more body fat in high-predation blue holes while females tended to have more body fat in low-predation blue holes; however, both males and females also exhibited strong among-population variation in this trait within predation regimes. Some of this variation could be explained by differences in resource availability within predation regimes, as well as differences among certain demographic variables (e.g., sex ratio or overall population density).

Finally, we also uncovered significant variation in life histories of both sexes among populations within the same predation regime. For example, females from the high-predation blue hole Hard Mile produced offspring that were more similar in number and size to those of low-predation blue holes than to the other high-predation blue holes. Similarly, males from the low-predation blue holes

Hubcap and Rainbow invested as much into reproduction as males from the seven high-predation blue holes. Such within-predation regime variation may be related to factors such as relative predation intensity, demography, time since colonization, genetic architecture, or sexual selection, and await future study.

Poeciliids are characterized by pronounced sexual dimorphism (Farr 1989), and congruently, "sex" had the strongest effect in the combined multivariate model, being driven by differences in "reproductive investment" (RA for females, GSI for males) and SL. In agreement with previous studies (Farr 1989), males were smaller than females and invested proportionally less into reproduction. In fact, the large investment into reproduction by female poeciliids leads to a visible distension of the abdomen that can even result in impaired maneuverability and predator evasion, while a similar phenomenon is not known for males (e.g., Plaut 2002; Ghalambor et al. 2004; Belk and Tuckfield 2010; Langerhans and Reznick 2010; Plath et al. 2011). However, it is important to keep in mind that measuring GSI for males and RA for females will capture only a fraction of the total investment into reproduction. Other costs of reproduction (i.e., energetic costs related to searching for mates, courtship, sneaking, and intrasex aggression) will undoubtedly also be important (Andersson 1994). This is particularly true for males, where the relative behavioral costs of reproduction may even exceed that captured by GSI (e.g., cost of sperm less than cost of eggs/embryos). Thus, patterns in total reproductive costs may not necessarily mirror those observed for GSI and RA per se, although behavioral costs in males, as measured by the frequency of sexual behaviors and intrasex aggression, are also elevated in high-predation populations (Heinen et al., unpublished manuscript).

Matrotrophy Index and the Test of the Trexler-DeAngelis Model

While substantial matrotrophy in poeciliids seems to be associated with the evolution of a so-called "follicular pseudoplacenta" (reviewed by Pires et al. 2011), species without a pseudoplacenta are often capable of at least incipient matrotrophy (Marsh-Matthews 2011; Pires et al. 2011). Our data demonstrate that *G. hubbsi*, a species lacking a follicular pseudoplacenta (Pollux et al. 2009), is capable of varying degrees of maternal provisioning as indicated by MI values covering almost the full range of what is traditionally interpreted as the continuum between lecithotrophy and incipient matrotrophy (Marsh-Matthews 2011; Pires et al. 2011). In fact, our data provide the first evidence for a matrotrophic signal in the genus *Gambusia* based on evaluation of the matrotrophy index alone (Pollux et al. 2009). Previous studies reporting on incipient matrotrophy in this genus relied on tracing radioactively labeled amino acids to detect low levels of postfertilization maternal provisioning (e.g., Marsh-Matthews et al. 2005; Marsh-Matthews and Deaton 2006; Marsh-Matthews 2011).

Our data strongly support the two predictions of the Trexler-DeAngelis (2003) model, because *G. hubbsi* females (1) generally had higher postfertilization maternal provisioning rates at higher values of environmental resource availability, (2) in general are capable of embryo abortion, (3) appear to abort on a somewhat regular basis, and (4) have higher abortion rates in low-resource environments (although this particular result is relatively weak and could have benefitted from a larger sample size). Our data did not, however, provide particularly strong support for the model's assumptions, suggesting that the model might be applicable over a more general set of fitness-related responses than originally modeled. We found no evidence for a between-population association between MI values and body condition or fecundity; although we did find suggestive evidence that populations with higher MI values tended to produce smaller oocytes. Our results from field-caught *G. hubbsi* inhabiting blue holes that differ in resource availability independent of predation regime thus strengthen the support for resource availability as one of the main environmental factors driving the evolution of matrotrophy (see also Trexler 1997; Marsh-Matthews and Deaton 2006).

With regard to abortion incidence, most studies have so far not supported the prediction of the Trexler-DeAngelis model (e.g., Marsh-Matthews and Deaton 2006; Banet and Reznick 2008; Banet et al. 2010; but see Trexler 1997). However, it is important to keep in mind that the Trexler-DeAngelis model did not predict abortion rates to generally be higher in matrotrophic species but rather that during periods of resource shortage, matrotrophic females should exhibit higher rates of abortion in an attempt to redistribute resources to a select subclutch. Furthermore, abortion was only important under the models' key assumption that matrotrophic reproduction might lead to females investing in reproduction beyond their energetic capacity to complete the brood; in other words if matrotrophic females also had a higher fecundity than lecithotrophic females (Trexler and DeAngelis 2003). This assumption clearly was not met by our data on *G. hubbsi* as fecundity did not differ as a function of the degree of matrotrophy. Thus our data are not suitable to directly test this particular prediction, although our data support the notion that species capable of at least incipient matrotrophy are capable of abortion and commonly abort embryos, especially in resource-poor environments (see also Marsh-Matthews and Deaton 2006).

This raises the question why previous studies on species relying on substantial matrotrophy (or placentotrophy), were unable to find evidence for abortion in these species under low-resource conditions (e.g., Banet and Reznick 2008; Banet et al. 2010). We hypothesize that the slightly contradictory results could indicate that the Trexler-DeAngelis model is better suited to explain the evolution of incipient matrotrophy from lecithotrophy, while the evolution of substantial matrotrophy (and thus the evolution of placentas) from incipient matrotrophy might be better explained by parent-offspring conflict as proposed by Crespi and Semeniuk (2004; see also discussion in Banet et al. 2010).

Finally, our finding that the degree of maternal provisioning did not affect patterns of female condition or fecundity (and only marginally influenced oocyte size) appear to be indicative of an important trade-off. Our multivariate analyses on life-history strategies in this system revealed that predation appears to be the dominant selective agent (see also Riesch et al., unpublished manuscript), while other between-population differences played a lesser role in explaining patterns of life-history evolution. Thus it is possible that selection on the offspring size/fecundity trade-off and female condition as a result of divergent predation regimes simply overrides weaker selection as a result of the degree of maternal provisioning.

Taken together with other studies that outline trait divergence in behavior (Langerhans et al. 2007; Heinen et al. unpublished manuscript), genetics (Langerhans et al. 2007), life history (Downhower et al. 2000, 2002), morphology (Langerhans et al. 2005, 2007), pigmentation (R. A. Martin et al., unpublished manuscript), and physiological performance (Langerhans 2009), our study strongly supports the notion of incipient ecological speciation among Bahamas mosquitofish populations as a response to divergent predation regimes.

Acknowledgments

We thank R. Albury and the Department of Fisheries of the Bahamas government for permission to conduct this work; B. Bohl and the Forfar field station for support in the field; C. Hess for help with data acquisition; and M. Belk and B. Schaalje for assistance with mixed-model multivariate analysis of variance. J. Trexler and an anonymous reviewer helped improve a previous version of the manuscript. Funding came from the National Science Foundation of the United States (DEB-0842364) and the W. M. Keck Center for Behavioral Biology at North Carolina State University.

Literature Cited

Abrams, P. A., and L. Rowe. 1996. The effects of predation on the age and size of maturity of prey. Evolution 50:1052–1061.

Andersson, M. 1994. Sexual selection. Princeton University Press, Princeton, NJ.

Banet, A. I., A. G. Au, and D. N. Reznick. 2010. Is mom in charge? implications of resource provisioning on the evolution of the placenta. Evolution 64:3172–3182.

Banet, A. I., and D. N. Reznick. 2008. Do placental species abort offspring? testing an assumption of the Trexler-DeAngelis model. Functional Ecology 22:323–331.

Belk, M. C., and R. C. Tuckfield. 2010. Changing costs of reproduction: age-based differences in reproductive allocation and escape performance in a livebearing fish. Oikos 119:163–169.

Blackburn, D. G. 1992. Convergent evolution of viviparity, matrotrophy, and specializations for fetal nutrition in reptiles and other vertebrates. American Zoologist 32:313–321.

———. 1999. Viviparity and oviparity: evolution and reproductive strategies. Pages 994–1003 *in* T. E. Knobil and J. D. Neill, eds. Encyclopedia of reproduction. Academic Press, New York.

———. 2005. Amniote perspectives on the evolutionary origins of viviparity and placentation. Pages 301–322 *in* H. Grier and M. C. Uribe, eds. Viviparity in fishes. New Life, Homestead, FL.

Bronikowski, A. M., M. E. Clark, F. H. Rodd, and D. N. Reznick. 2002. Population-dynamic consequences of predator-induced life history variation in the guppy (*Poecilia reticulata*). Ecology 83: 2194–2204.

Cohen, J. E. 1988. Statistical power analysis for the behavioral sciences. 2nd ed. Earlbaum, Hillsdale, NJ.

Crespi, B., and C. Semeniuk. 2004. Parent-offspring conflict in the evolution of vertebrate reproductive mode. American Naturalist 163:635–653.

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

———. 2002. Litter overlap in *Gambusia hubbsi*: superfetation revisited. Environmental Biology of Fishes 65:423–430.

Endler, J. A. 1986. Natural selection in the wild. Princeton University Press, Princeton, NJ.

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

Fairbanks, R. G. 1989. A 17000-year glacio-eustatic sea level record: influence of glacial melting rates on the Younger Dryas event and deep-ocean circulation. Nature 342:637–642.

Farr, J. A. 1989. Sexual selection and secondary sexual differentiation in poeciliids: determinants of male mating success and the evolution of female choice. Pages 91–123 *in* G. K. Meffe and F. F. Snelson Jr., eds. Ecology & Evolution of Livebearing Fishes (Poeciliidae). Prentice Hall, Englewood Cliffs, NJ.

Gadgil, M., and W. H. Bossert. 1970. Life historical consequences of natural selection. American Naturalist 104:1–24.

Ghalambor, C. K., D. N. Reznick, and J. A. Walker. 2004. Constraints on adaptive evolution: the functional trade-off between reproduction and fast-start swimming performance in the Trinidadian guppy (*Poecilia reticulata*). American Naturalist 164:38–50.

Gluckman, T. L., and K. B. Hartney. 2000. A trophic analysis of mosquitofish, *Gambusia hubbsi* Breder, inhabiting blue holes on Andros Island, Bahamas. Caribbean Journal of Science 36:104–111.

Grether, G. F., and G. R. Kolluru. 2011. Evolutionary and plastic responses to resource availability. Pages 61–71 *in* J. P. Evans, A. Pilastro, and I. Schlupp, eds. Ecology and evolution of Poeciliid fishes. University of Chicago Press, Chicago.

Hendry, A. P., M. L. Kelly, M. T. Kinnison, and D. N. Reznick. 2006. Parallel evolution of the sexes? effects of predation and habitat features on the size and shape of wild guppies. Journal of Evolutionary Biology 19:741–754.

Heulett, S. T., S. C. Weeks, and G. K. Meffe. 1995. Lipid dynamics and growth relative to resource level in juvenile eastern mosquitofish (*Gambusia holbrooki*: Poeciliidae). Copeia 1995:97–104.

Hrbek, T., J. Seckinger, and A. Meyer. 2007. A phylogenetic and biogeographic perspective on the evolution of poeciliid fishes. Molecular Phylogenetics and Evolution 43:986–998.

Jennions, M. D., and S. R. Telford. 2002. Life-history phenotypes in populations of *Brachyrhaphis episcopi* (Poeciliidae) with different predator communities. Oecologia (Berlin) 132:44–50.

Jennions, M. D., B. B. M. Wong, A. Cowling, and C. Donnelly. 2006. Life-history phenotypes in a live-bearing fish *Brachyrhaphis episcopi* living under different predator regimes: seasonal effects? Environmental Biology of Fishes 76:211–219.

Johnson, J. B. 2001. Adaptive life-history evolution in the livebearing fish *Brachyrhaphis rhabdophora*: genetic basis for parallel divergence in age and size at maturity and a test of predator-induced plasticity. Evolution 55:1486–1491.

———. 2002. Divergent life histories among populations of the fish *Brachyrhaphis rhabdophora*: detecting putative agents of selection by candidate model analysis. Oikos 96:82–91.

Johnson, J. B., and J. C. Bagley. 2011. Ecological drivers of life-history divergence. Pages 38–49 *in* J. P. Evans, A. Pilastro, and I. Schlupp, eds. Ecology and evolution of poeciliid fishes. University of Chicago Press, Chicago.

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

Kallman, K. D., and M. P. Schreibman. 1973. A sex-linked gene controlling gonadotrop differentiation and its significance in determining the age of sexual maturation and size of platyfish, *Xiphophorus maculatus*. General and Comparative Endocrinology 21: 287–304.

Kenward, M. G., and J. H. Roger. 1997. Small sample inference for fixed effects from restricted maximum likelihood. Biometrics 53: 983–997.

Kozlowski, J., and J. Uchmansky. 1987. Optimal individual growth and reproduction in perennial species with indeterminate growth. Evolutionary Ecology 1:231–244.

Langerhans, R. B. 2009*a*. Morphology, performance, fitness: functional insight into a post-Pleistocene radiation of mosquitofish. Biology Letters 5:488–491.

———. 2009*b*. Trade-off between steady and unsteady swimming underlies predator-driven divergence in *Gambusia affinis*. Journal of Evolutionary Biology 22:1057–1075.

Langerhans, R. B., and T. J. DeWitt. 2004. Shared and unique features of evolutionary diversification. American Naturalist 164:335–349.

Langerhans, R. B., M. Gifford, and E. Joseph. 2007. Ecological speciation in *Gambusia* fishes. Evolution 61:2056–2074.

Langerhans, R. B., C. A. Layman, and T. J. DeWitt. 2005. Male genital size reflects a trade-off between attracting mates and avoiding predators in two live-bearing fish species. Proceedings of the National Academy of Sciences of the USA 102:7618–7623.

Langerhans, R. B., C. A. Layman, A. M. Shokrollahi, and T. J. DeWitt. 2004. Predator-driven phenotypic diversification in *Gambusia affinis*. Evolution 58:2305–2318.

Langerhans, R. B., and A. M. Makowicz. 2009. Shared and unique features of morphological differentiation between predator regimes in *Gambusia caymanensis*. Journal of Evolutionary Biology 22: 2231–2242.

Langerhans, R. B., and D. N. Reznick. 2010. Ecology and evolution of swimming performance in fishes: predicting evolution with biomechanics. Pages 200–248 *in* P. Domenici and B. G. Kapoor, eds. Fish locomotion: an etho-ecological perspective. Science, Enfield, NH.

Law, R. 1979. Optimal life histories under age-specific predation. American Naturalist 114:319–417.

Magurran, A. E. 2005. Evolutionary ecology: the Trinidadian guppy. Oxford University Press, Oxford.

Marsh-Matthews, E. 2011. Matrotrophy. Pages 18–27 *in* J. P. Evans, A. Pilastro, and I. Schlupp, eds. Ecology and evolution of poeciliid fishes. University of Chicago Press, Chicago.

Marsh-Matthews, E., M. Brooks, R. Deaton, and H. Tan. 2005. Effects of maternal and embryo characteristics on post-fertilization provisioning in fishes of the genus *Gambusia*. Oecologia (Berlin) 144: 12–24.

Marsh-Matthews, E., and R. Deaton. 2006. Resources and offspring provisioning: a test of the Trexler-DeAngelis model for matrotrophy evolution. Ecology 87:3014–3020.

Martin, T. E. 1995. Avian life history evolution in relation to nest sites, nest predation, and food. Ecological Monographs 65:101–127.

Mattingly, H. T., and M. J. Butler IV. 1994. Laboratory predation on the Trinidadian guppy: implications for the size-selective predation hypothesis and guppy life history evolution. Oikos 69:54–64.

Michod, R. E. 1979. Evolution of life histories in response to age-specific mortality factors. American Naturalist 113:531–550.

Olsson, M., R. Shine, and E. Bak-Olsson. 2000. Locomotor impairment of gravid lizards: is the burden physical or physiological? Journal of Evolutionary Biology 13:263–268.

Ostrovsky, A. N., D. P. Gordon, and S. Lidgard. 2009. Independent evolution of matrotrophy in the major classes of Bryozoa: transitions among reproductive patterns and their ecological background. Marine Ecology Progress Series 378:113–124.

Pires, M. N., A. I. Banet, B. J. A. Pollux, and D. N. Reznick. 2011. Variation and evolution of reproductive strategies. Pages 28–37 *in* J. P. Evans, A. Pilastro, and I. Schlupp, eds. Ecology and evolution of poeciliid fishes. University of Chicago Press, Chicago.

Pires, M. N., K. E. McBride, and D. N. Reznick. 2007. Interpopulation variation in life-history traits of *Poeciliopsis prolifica*: implications for the study of placental evolution. Journal of Experimental Zoology 307A:113–125.

Plath, M., R. Riesch, Z. W. Culumber, B. Streit, and M. Tobler. 2011. Giant water bug (*Belostoma* sp.) predation on a cave fish (*Poecilia mexicana*): effects of female body size and gestational state. Evolutionary Ecology Research 13:133–144.

Plaut, I. 2002. Does pregnancy affect swimming performance of female mosquitofish, *Gambusia affinis*? Functional Ecology 16:290–295.

Pollux, B. J. A., M. N. Pires, A. I. Banet, and D. N. Reznick. 2009. Evolution of placentas in the fish family Poeciliidae: an empirical study of macroevolution. Annual Review of Ecology and Systematics 40:271–289.

Pollux, B. J. A., and D. N. Reznick. 2011. Matrotrophy limits a female's ability to adaptively adjust offspring size and fecundity in fluctuating environments. Functional Ecology 25:747–756.

Reznick, D. 1981. "Grandfather effects": the genetics of interpopulation differences in offspring size in the mosquito fish. Evolution 35:941–1020.

Reznick, D. N., H. A. Bryga, and J. A. Endler. 1990. Experimentally induced life-history evolution in a natural population. Nature 346: 357–359.

Reznick, D. N., M. J. Butler IV, F. H. Rodd, and P. Ross. 1996*a*. Life-history evolution in guppies (*Poecilia reticulata*). 6. Differential mortality as a mechanism for natural selection. Evolution 50:1651–1660.

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

Reznick, D. N., M. Mateos, and M. S. Springer. 2002. Independent origins and rapid evolution of the placenta in the fish genus *Poeciliopsis*. Science 298:1018–1020.

Reznick, D., A. Meyer, and D. Frear. 1993. Life history of *Brachyraphis rhabdophora* (Pisces: Poeciliidae). Copeia 1993:103–111.

Reznick, D. N., F. H. Rodd, and M. Cardenas. 1996*b*. Life-history evolution in guppies (*Poecilia reticulata*: Poeciliidae). IV. Parallelism in life-history phenotypes. American Naturalist 147:319–338.

Riesch, R., M. Plath, and I. Schlupp. 2011*a*. Toxic hydrogen sulphide and dark caves: pronounced male life-history divergence among locally adapted *Poecilia mexicana* (Poeciliidae). Journal of Evolutionary Biology 24:596–606.

Riesch, R., M. Plath, I. Schlupp, and E. Marsh-Matthews. 2010. Matrotrophy in the cave molly: an unexpected provisioning strategy in an extreme environment. Evolutionary Ecology 24:789–801.

Riesch, R., I. Schlupp, R. B. Langerhans, and M. Plath. 2011*b*. Shared and unique patterns of embryo development in extremophile poeciliids. PLoS ONE 6:e27377.

Roff, D. A. 2002. Life history evolution. Sinauer, Sunderland, MA.

Schreibman, M. P., and K. D. Kallman. 1977. The genetic control of the pituitary-gonadal axis in the platyfish, *Xiphophorus maculatus*. Journal of Experimental Zoology 200:277–294.

Schug, M. D., J. F. Downhower, L. P. Brown, D. B. Sears, and P. A. Fuerst. 1998. Isolation and genetic diversity of *Gambusia hubbsi* (mosquitofish) populations in blueholes on Andros Island, Bahamas. Heredity 80:336–346.

Scrimshaw, N. S. 1945. Embryonic development in poeciliid fishes. Biological Bulletin 88:233–246.

Spitze, K. 1991. *Chaoborus* predation and life-history evolution in *Daphnia pulex*: temporal pattern of population diversity, fitness, and mean life history. Evolution 45:82–92.

Stearns, S. C. 1992. The evolution of life histories. Oxford University Press, New York.

Stibor, H. 1992. Predator induced life-history shifts in a freshwater cladoceran. Oecologia (Berlin) 92:162–165.

Thompson, M. B., J. R. Stewart, B. K. Speake, M. J. Hosie, and C. R. Murphy. 2002. Evolution of viviparity: what can Australian lizards tell us? Comparative Biochemistry and Physiology B 131: 631–643.

Trexler, J. C. 1997. Resource availability and plasticity in offspring provisioning: embryo nourishment in sailfin mollies. Ecology 78: 1370–1381.

Trexler, J. C., and D. L. DeAngelis. 2003. Resource allocation in offspring provisioning: an evaluation of the conditions favoring the evolution of matrotrophy. American Naturalist 162:574–585.

Trexler, J. C., D. L. DeAngelis, and J. Jiang. 2011. Community assembly and mode of reproduction: predicting the distribution of livebearing fishes. Pages 95–108 *in* J. P. Evans, A. Pilastro, and I. Schlupp, eds. Ecology and evolution of poeciliid fishes. University of Chicago Press, Chicago.

Turner, C. L. 1941. Morphogenesis of the gonopodium in *Gambusia affinis affinis*. Journal of Morphology 69:161–185.

Walker, J. A., C. K. Ghalambor, O. L. Griset, D. McKenney, and D. N. Reznick. 2005. Do faster starts increase the probability of evading predators? Functional Ecology 19:808–815.

Wourms, J. P. 1981. Viviparity: the maternal-fetal relationship in fishes. American Zoologist 21:473–515.

Associate Editor: Suzanne Henson Alonzo
Editor: Mark A. McPeek

Male *Gambusia hubbsi* from East Twin (low predation). Photo by R. Brian Langerhans.