



# Habitat predicts reproductive superfetation and body shape in the livebearing fish *Poeciliopsis turrubarensis*

J. Jaime Zúñiga-Vega, David N. Reznick and Jerald B. Johnson

J. J. Zúñiga-Vega and J. B. Johnson ([jerry.johnson@byu.edu](mailto:jerry.johnson@byu.edu)), Dept of Integrative Biology, Brigham Young Univ., Provo, UT 84602, USA. – D. N. Reznick, Dept of Biology, Univ. of California, Riverside, CA 92521, USA.

Superfetation, the ability of females to simultaneously carry more than one brood at different developmental stages, is an unusual reproductive strategy that has independently evolved several times in the livebearing fish family Poeciliidae. Why this strategy has evolved remains uncertain. One hypothesis is that superfetation is a response to selective pressures that constrain the physical space within a female in which her offspring can develop. This hypothesis is reasonable, because superfetation should reduce the total volume needed to house developing embryos – that is, fewer large, fully developed embryos will be held by a superfetating female (with several broods at different developmental stages) than a non-superfetating female (where all embryos reach a fully developed stage at the same time).

In this study, we explore this ‘morphological constraint’ hypothesis of superfetation by examining the livebearing fish, *Poeciliopsis turrubarensis*. We found that populations vary markedly in degree of superfetation, with individuals carrying from two to four distinct broods across different geographic areas. These populations also occupy a range of habitat types: some populations occur in slow moving coastal rivers near the ocean, while other populations occur far inland in fast moving waters that drain steep mountain environments. In comparing populations from these two types of environments, we find a strong association between stream habitat type and the degree of superfetation within populations. Fish from inland populations have higher levels of superfetation than their coastal counterparts. In addition, geometric morphometric analysis revealed that inland populations are also more fusiform than fish from coastal locations. Combined, these two lines of evidence support the ‘morphological constraint’ hypothesis, and suggest that the life history strategy of superfetation could be driven by environmental pressures that favor a more streamlined phenotype.

One of the most remarkable patterns of diversity in nature is the variety of strategies that organisms use to reproduce. Several ecological factors are known to directly shape reproductive traits – examples include predator-mediated mortality, density-dependent competition, and resource availability (reviewed by Roff 1992, 2002, Stearns 1992). Reproductive traits can also be influenced by an organism’s phenotype. For example, morphological constraints frequently dictate the kinds of reproductive options that a species can employ (Glazier 2000, Beck and Beck 2005). Hence, ecological factors not only have the potential to directly shape reproduction, but by influencing morphology can also have an important indirect effect on reproduction.

Despite our growing understanding of factors that can influence reproductive evolution, some life history

strategies remain a puzzle for evolutionary ecologists to explain. Superfetation, the ability of a female to simultaneously carry several broods at different developmental stages (Turner 1937, 1940, Scrimshaw 1944) is an unusual reproductive mode that has been documented in several different taxa, including mammals (Yamaguchi et al. 2004, 2006), angiosperm plants (Kennedy 1978), and most predominately, in livebearing fishes (Thibault and Schultz 1978). The phylogenetic distribution of superfetation within the livebearing fish family Poeciliidae suggests that in this group alone it has evolved independently at least four times (Reznick and Miles 1989). Yet, it remains unclear why this strategy has evolved.

Among ideas that have emerged to explain the origins of this phenomenon in fishes, the most

prominent suggests it is a response to morphological constraints imposed by maintaining a streamlined phenotype in an aqueous environment (Thibault and Schultz 1978, Reznick and Miles 1989). In other words, because the physical space available to carry offspring cannot increase indefinitely in a female without some negative effect on swimming performance, a female becomes constrained to a body shape (and volume) that restricts the space she can partition to offspring. In livebearing fishes, this constraint is amplified as developing embryos take on water resulting in a two- to three-fold increase in volume and wet mass (Ghalambor et al. 2004). Superfetation offers a solution to this dilemma in that it allows a female to carry fewer large, full-term embryos at any given time. The net effect is that superfetating females should be able to produce the same number of offspring as their non-superfeting counterparts, but do so with a more streamlined phenotype. Hence, we expect superfetation (i.e. the number of broods carried) to increase in response to any environmental selective pressure that favors a streamlined phenotype (e.g. in high stream velocity environments, or in habitats where fish must swim quickly to escape predators).

Most attempts to understand the evolution of superfetation have focused on differences among species (Thibault and Schultz 1978, Reznick and Miles 1989, Meisner and Burns 1997). However, intraspecific comparisons may hold more promise because populations are more likely to occupy the selective environments actually responsible for phenotypic divergence (Travis et al. 1987, Downhower et al. 2002). To date, no studies have tested for the expected relationship among stream velocity, morphological differences, and variation in the degree of superfetation, despite several livebearing fishes that employ this reproductive strategy. What is required to conduct this test is a widespread, abundant species that occupies a range of selective environments and that shows variation in superfetation among populations.

Here, we show that the livebearing fish *Poeciliopsis turrubarensis* meets these criteria thereby providing a valuable model to test the 'morphological constraint' hypothesis to explain superfetation. Populations show considerable variation in the degree of superfetation, with females from some localities having up to four broods at distinct developmental stages and others having as few as two broods. Populations also occupy habitats that range from almost still water to those with high stream velocity where a streamlined phenotype should be favored. In addition, this species is abundant throughout its range making it easy work with in the field. It also has a short generation time and can be maintained in the laboratory, making it possible to keep in captivity and to utilize for manipulative experiments (Bussing 1987, Cabrera-Peña and Solano-López 1995).

Hence, in this study we used *P. turrubarensis* to explore the ecological conditions and morphological constraints associated with superfetation. Specifically, we focused on three objectives: (1) to describe variation in superfetation and other related life history traits among several distinct populations of *P. turrubarensis* in Central America; (2) to test the 'morphological constraint' hypothesis by comparing levels of superfetation between populations from still water sites vs those from high velocity sites; and (3) to use geometric morphometric techniques to explore if superfetation is associated with a concomitant shift in body morphology reflecting a more streamlined phenotype. Our results show that superfetation is higher in high velocity environments than in still water environments and that pregnant female fish from these populations are in fact more fusiform than their still-water counterparts.

## Material and methods

### Study system and collection sites

*Poeciliopsis turrubarensis* (Poeciliidae) is distributed from southern Mexico to central Colombia. Here we focus on populations from Costa Rica that occur in rivers that drain from the central mountain range westward to the Pacific Ocean (Fig. 1). This species is common in coastal environments, particularly in slow moving waters near the mouths of rivers. However, several populations are found further inland (elevations up to 120 m; Bussing 1987) at much steeper stream gradients than coastal environments. These inland habitats present a high water-velocity environment where selection should favor more streamlined phenotypes than in coastal environments where water velocity is almost still. In turn, we expect selection for streamlining to result in higher levels of superfetation, thereby allowing females from inland environments to produce as many offspring as their coastal counterparts, but without requiring as much physical volume to do so.

In this study we divide *P. turrubarensis* habitats into two kinds: coastal sites, located within 5 km of the ocean; and inland sites, located more than 5 km from the ocean. Our 5 km boundary delineates a marked shift in stream gradient from low gradient coastal habitats to high gradient inland habitats. Average stream slope was  $3.43 \pm 0.642$  m km<sup>-1</sup> SE for coastal sites and  $17.67 \pm 3.141$  m km<sup>-1</sup> SE for inland sites ( $t_{10} = -4.441$ ,  $p = 0.001$ ). We recognize that these habitat types might differ in ecological characteristics other than stream gradient, and we interpret our findings in light of this possibility (Discussion). However, because our goal was to examine the relationship between stream velocity, female reproductive

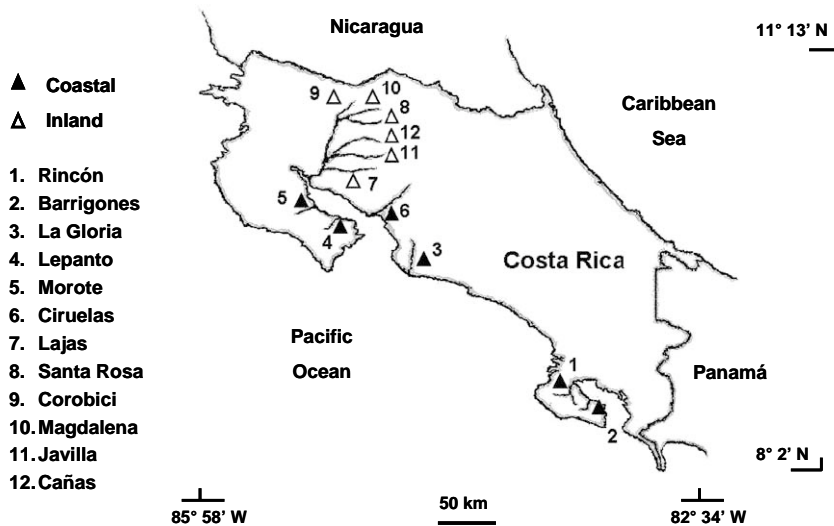


Fig. 1. Geographic locations of the 12 populations of *Poeciliopsis turrubarensis* included in this study (geographic coordinates available upon request). Names for populations were assigned according to the river drainages from which samples were extracted.

morphology, and superfetation, we chose our collecting sites a priori to emphasize the inland vs coastal contrast.

We collected fish from 12 locations: six inland populations vs six coastal populations (Fig. 1). All collections were made during the dry season (January to March) between 1997 and 2006. We examined the effect of 'year' on the measured life-history traits (variables defined below) by comparing samples made from the same location over multiple years. In most cases, the year of collection had no effect on the observed life history patterns and we pooled these data by location across years. However, two sites (Lajas and Santa Rosa) showed variation among years that can be ascribed to small sample sizes at these sites in 1997 and 1998; hence, we discarded these data from the analysis. Fish from each site were collected exactly the same way, using a hand-held seine net (1.3 m depth  $\times$  5 m length; 8 mm mesh size). At inland sites, fish were collected from the main current and in areas adjacent to it; at coastal sites, fish were most common in areas where currents slowed as streams approached the ocean. Immediately following collection, all fish were preserved in 100% ethanol. We attempted to collect around 200 individuals per location, providing us with enough mature females to accurately measure superfetation and other life history traits in each population.

### Quantifying superfetation and life history traits

In this study we examined variation in six reproductive traits: (1) number of broods per female (superfetation);

(2) number of embryos per brood; (3) size of individual embryos; (4) reproductive investment per brood; (5) total reproductive investment; and (6) matrotrophy index. Although the degree of superfetation is part of an overall life history strategy and most likely integrated with other life history traits, we have treated superfetation as a potentially independent character. This decision stems from a fundamental observation: changes in the degree of superfetation need not impact the rate of offspring production or the amount of energy invested in reproduction. Instead, variation in superfetation simply alters the distribution of offspring production in time (Thibault and Schultz 1978). Consequently our hypotheses and our analyses are construed to allow the possibility that superfetation could vary independently of other elements of the fish life history.

We quantified reproductive traits following methods described in Reznick et al. (1993) and in Johnson and Belk (2001). In brief, superfetation is defined as the average number of broods an adult female carries at any given time. We calculated superfetation for each population by counting the number of broods per female. Broods were defined according to developmental stage following the 11-stage classification method of Haynes (1995). In no cases did we have difficulty distinguishing between broods using this scheme. Number of embryos per brood was counted directly from each dissected female. Embryo size was calculated by drying an entire brood of offspring in a desiccating oven (24 h at 55°C) and dividing brood dry mass by the number of individuals in that brood. Reproductive

investment per brood was calculated as the dry brood mass divided by the dry mass of the female (below), whereas total reproductive investment was estimated as the summed dry mass of all the broods present in a female divided by the dry mass of the female. Finally, a matrotrophy index (MI) was calculated for each population by dividing the estimated dry mass of the embryo just prior to birth (stage 11 following Haynes 1995) by the dry mass of the egg just following fertilization (stage 4). The matrotrophy index is a measure of the degree of maternal provisioning to the embryos, with higher MI values indicative of greater maternal investment (Wourms et al. 1988). Almost all livebearing fish with superfetation show matrotrophy (Reznick and Miles 1989, but see Lima 2005), yet it is not clear if there is an association between superfetation and matrotrophy within a single species.

In addition to the six reproductive traits listed above, we also measured the dry mass of each adult female (desiccated with intestine removed); this measure was used as a covariate in the statistical analyses described below. To meet assumptions of these statistical analyses we made the following data transformations to the life history traits: the count data of number of broods per female and number of individuals per brood were square-root transformed; individual embryo dry mass, reproductive investment per brood, total reproductive investment, and female dry mass were  $\log_{10}$  transformed.

### Quantifying shape variation using geometric morphometrics

We quantified variation in body shape among *P. turrubarensis* populations using digital images of preserved reproductive females. Digital photos were taken before females were dissected. We examined the lateral profile of each female by scoring 18 anatomical landmarks (landmark locations available upon request). To eliminate possible shape bias associated with ontogenetic development, juvenile females were not included. Using two-dimensional landmarks we computed a set of "shape variables" for each fish using the thin-plate spline approach (Zelditch et al. 2004) implemented in the program TPSRELW (Rohlf 2002, <http://life.bio.sunysb.edu/morph>). This software calculates two measures of shape variation for each specimen: a set of uniform shape components, which describe changes in form that are geometrically uniform across the entire body of the fish (e.g. general increase in length or width relative to an average or consensus shape), and a set of partial warps or non-uniform shape components, which describe non-uniform changes in the position of a subset of landmarks relative to other landmarks (Zelditch et al. 2004). Using the same

software, we calculated relative warp (RW) scores from the uniform and non-uniform shape variables. Relative warps are orthogonal axes of shape variation generated from a principal components analysis that can be used to interpret changes in body shape (Johnson et al. 2004). These RW scores also serve as the input variables for statistical analyses comparing inland vs coastal populations (described below). The thin-plate spline approach also allowed us to graph deformations from the average shape (among all individuals and across all populations) for each fish, providing a graphical representation of shape differences between inland and coastal populations.

### Statistical analyses

To generate comparable estimates among populations for number of broods, number of embryos per brood, embryo size, reproductive investment per brood, and total reproductive investment, we adjusted these life history traits using analysis of covariance (ANCOVA). In each case, habitat type was included as a main effect. We also considered population nested within habitat type to test for variation among locations within coastal and inland environments. Female dry mass was included as a covariate to account for differences due to the size of the mother. In addition the following covariates were included. For number of embryos per brood we included number of broods per female; for embryo dry mass we included embryo developmental stage; and finally, for total reproductive investment we included stage of development for the most mature brood carried by the female.

In each of these analyses, we found a significant interaction between female dry mass and population; we also found a significant interaction between number of broods and population in the analysis for number of embryos per brood. However, we justified the inclusion of these covariates in the models for two reasons (*sensu* Reznick 1989): (1) the interactions were typically caused by one or two slopes whose removal caused the interaction to be non-significant but did not qualitatively alter our results; and (2) the covariates always explained a significant amount of the total variation in the models.

The biological phenomenon of superfetation presented an interesting statistical challenge: broods derived from the same female are not independent observations. Rather, each sibling brood is nested within the same female. However, because females carried different numbers of broods, it was not possible to run a balanced nested ANCOVA that included all appropriate factors, covariates and interactions. Consequently, we tested for differences in life history traits in two ways. First, we randomly chose a single brood from

each female and ran the analyses with data from just one brood per female (the most conservative approach). We also ran the analyses with all broods included, essentially treating broods as if they were independent observations. The results of these two sets of analyses were qualitatively identical, providing a robust set of inferences irrespective of the nature of the analysis. For simplicity, we present only the results from the ‘all-brood’ analysis; results from the complementary set of analyses are available upon request. All life history analyses were executed using a generalized linear models procedure.

For our shape analysis we compared relative warp (RW) scores among populations and between coastal and inland sites using a nested multivariate analysis of variance (nested MANOVA). Individual RW scores were used as dependent variables and type of habitat and population (nested in habitat) were used as categorical factors. All analyses were executed in the statistical package STATISTICA 6.0 (Anonymous 2001).

## Results

*Poeciliopsis turrubarensis* populations show wide variation for each of the measured life history traits (Table 1). In addition to the six reproductive traits of interest, we also report average female dry mass and the maximum number of broods observed in each population.

### Habitat predicts superfetation

*Poeciliopsis turrubarensis* from inland habitats have higher levels of superfetation than their coastal counterparts (adjusted least squares means from GLM: inland =  $2.28 \pm 0.03$  broods SE, coastal =  $1.68 \pm 0.04$  broods SE;  $F_{1,478} = 9.50$ ,  $p = 0.002$ ; Table 2, Fig. 2a). Variation in superfetation ranged from a single brood in some females to as many as four broods in others. All but one of the inland populations showed a maximum number of broods equal to four, whereas in coastal sites only one population showed individuals with four broods (Table 1). The covariate female dry mass and source population were also significant factors in the model (Table 2).

### Life history variation among populations

Individuals from inland populations produce significantly more embryos per brood compared to those from coastal populations (adjusted least squares means:  $12.64 \pm 0.29$  SE vs  $3.55 \pm 0.30$  SE, respectively;  $F_{1,1027} = 8.16$ ,  $p < 0.01$ ; Table 2, Fig. 2b). In addition,

Table 1. Summary of measured life-history traits from 12 populations of *Poeciliopsis turrubarensis*. Values for number of broods, number of embryos per brood, individual embryo dry mass, reproductive investment per brood, and total reproductive investment are adjusted least square means from generalized linear models (GLM) reported in text.

Type of habitat	Population	n	Mean female dry mass (g)	No. of broods	Maximum no. of broods	Embryos per brood	Individual embryo dry mass (g)	Reproductive investment per brood	Total reproductive investment	Matrotrophy index
Coastal	Rincón	43	0.316	1.809	3	7.903	0.0013	0.034	0.063	1.055
	Barrigones	77	0.311	1.847	3	6.773	0.0015	0.033	0.064	1.016
	La Gloria	54	0.270	2.125	3	7.505	0.0014	0.035	0.073	1.100
	Lepanto	41	0.339	1.712	4	5.543	0.0014	0.026	0.045	1.231
	Morote	41	0.227	2.181	3	11.548	0.0014	0.054	0.112	0.964
	Ciruelas	13	0.126	1.487	2	5.478	0.0018	0.036	0.025	1.020
Inland	Lajas	86	0.260	2.608	4	7.820	0.0016	0.042	0.099	1.051
	Santa Rosa	34	0.278	1.973	3	6.321	0.0013	0.029	0.060	0.807
	Corobici	24	0.249	1.695	4	6.872	0.0013	0.031	0.073	0.814
	Magdalena	34	0.469	1.387	4	8.128	0.0010	0.033	0.057	1.504
	Javilla	40	0.251	2.635	4	7.490	0.0014	0.037	0.089	0.989
	Cañas	14	0.622	2.428	4	8.280	0.0013	0.034	0.118	1.068

Table 2. Results of the generalized linear model (GLM) analyses testing for differences in several life history traits, including: number of broods (the measure of superfetation), number of embryos per brood, individual embryo dry mass, reproductive investment per brood, and total reproductive investment. Populations were nested within type of habitat (coastal or inland; see text for details).

Effect	SS	DF	MS	F	p
<b>Number of broods</b>					
Type of habitat	0.30	1	0.30	9.50	0.002
Population (habitat)	1.09	10	0.11	3.46	<0.001
Female dry mass	5.16	1	5.16	163.95	<0.001
Population (habitat) × female dry mass	2.11	10	0.21	6.71	<0.001
Error	15.05	478	0.03		
<b>Number of embryos per brood</b>					
Type of habitat	3.19	1	3.19	8.16	0.004
Population (habitat)	13.15	10	1.31	3.36	<0.001
Female dry mass	154.82	1	154.82	396.04	<0.001
Number of broods	6.76	1	6.76	17.29	<0.001
Population (habitat) × female dry mass	28.65	10	2.87	7.33	<0.001
Population (habitat) × number of broods	9.44	10	0.94	2.42	0.008
Error	401.48	1027	0.39		
<b>Individual embryo dry mass</b>					
Type of habitat	0.08	1	0.08	5.24	0.022
Population (habitat)	0.41	10	0.04	2.62	0.004
Female dry mass	0.80	1	0.80	51.00	<0.001
Developmental stage	0.58	1	0.58	37.13	<0.001
Population (habitat) × female dry mass	0.60	10	0.06	3.82	<0.001
Error	16.29	1037	0.02		
<b>Reproductive investment per brood</b>					
Type of habitat	0.04	1	0.04	0.62	0.430
Population (habitat)	4.20	10	0.42	7.28	<0.001
Female dry mass	0.87	1	0.87	15.10	<0.001
Population (habitat) × female dry mass	2.83	10	0.28	4.89	<0.001
Error	59.95	1038	0.06		
<b>Total reproductive investment</b>					
Type of habitat	0.22	1	0.22	6.24	0.013
Population (habitat)	2.25	10	0.23	6.29	<0.001
Female dry mass	1.12	1	1.12	31.38	<0.001
Latest developmental stage	3.50	1	3.50	97.78	<0.001
Population (habitat) × female dry mass	1.79	10	0.18	5.01	<0.001
Error	17.09	477	0.04		

we found a statistically significant association between number of embryos per brood and female dry mass, number of broods per female, and source population (Table 2).

The size of individual embryos was significantly smaller in inland populations compared to embryo size from coastal locations (adjusted means:  $1.06 \pm 0.002$  mg SE vs  $1.84 \pm 0.001$  mg SE, respectively;  $F_{1,1037} = 5.24$ ,  $p = 0.02$ ; Table 2, Fig. 2c). Female dry mass, developmental stage, and source population also had a significant effect on the dry mass of individual embryos (Table 2).

Reproductive investment per brood did not differ between coastal and inland habitats (adjusted means:  $0.03 \pm 0.0013$  SE for inland sites and  $0.04 \pm 0.0012$  SE for coastal sites;  $F_{1,1038} = 0.62$ ,  $p = 0.43$ ; Table 2, Fig. 2d). Female dry mass and source population did have significant effects on this trait (Table 2). In contrast to reproductive investment per brood, overall reproductive

investment (total mass of all broods per female) did differ between inland and coastal habitats. Populations from inland environments had higher values for this trait than their coastal counterparts (adjusted means:  $0.08 \pm 0.0016$  SE vs  $0.05 \pm 0.0017$  SE, respectively;  $F_{1,477} = 6.24$ ,  $p = 0.01$ ; Table 2, Fig. 2e).

Matrotrophy index varied from 0.807 to 1.504 among populations (Table 1). However, no significant difference was found between coastal and inland populations (coastal sites =  $1.06 \pm 0.04$  SE, inland sites =  $1.04 \pm 0.10$  SE;  $t_{10} = 0.23$ ,  $p = 0.82$ ).

### Habitat predicts body shape

Individuals from coastal and inland sites differ markedly in morphology as measured by relative warps (Wilk's  $\lambda = 0.77$ ,  $p < 0.001$ ). Fish from inland populations are more streamlined than are fish from coastal locations (Fig. 3; description below). We also found a

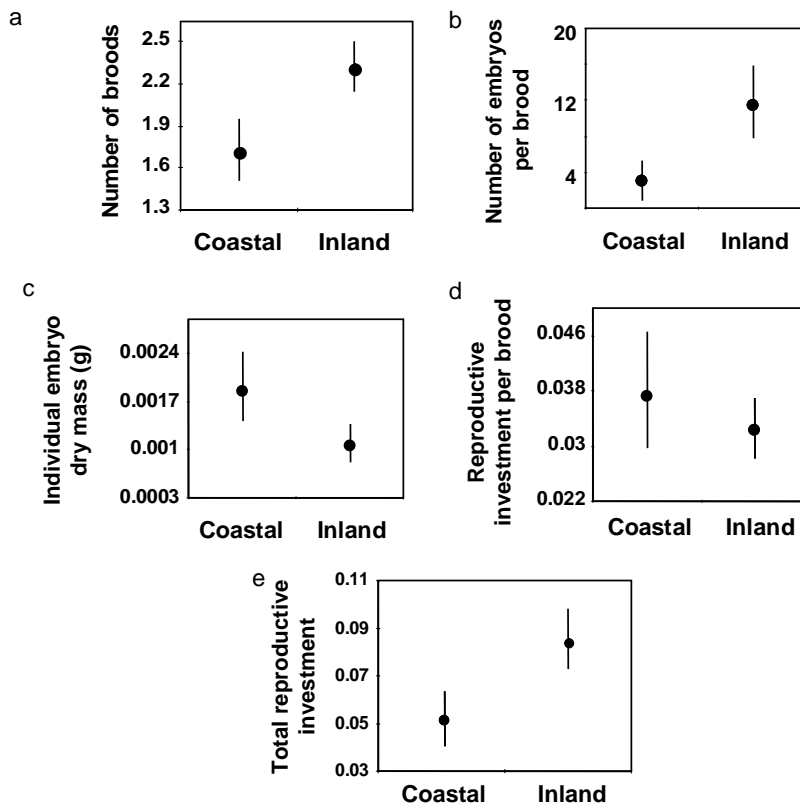


Fig. 2. Comparisons of five life history traits for populations from coastal habitats versus those from inland habitats. Data presented are adjusted least square means from the GLM analyses: (a) number of broods (superfetation); (b) number of embryos per brood; (c) individual embryo dry mass; (d) reproductive investment per brood; and (e) total reproductive investment. Vertical lines represent 95% confidence intervals.

statistical effect of source population upon the RW scores (Wilk's  $\lambda = 0.12$ ,  $p < 0.001$ ). Striking differences between coastal and inland sites in body shape are apparent when comparing deformation grids generated by the main scores in the relative warps of individuals within these two categories (Fig. 3).

Shape differences along the first three relative warps explained 48% of the total variance. In the first relative warp (21% of explained variance) coastal populations are marked by a relative shortening of the entire body and a relative broadening of the region between the abdomen and the dorsal fin (Fig. 3a). In contrast, inland populations show a lengthening and narrowing of the body with a notably shorter caudal peduncle (Fig. 3b). Deformations obtained from scores in the second relative warp (16% of explained variance) depict an artifact generated by including images of bent individuals (Fig. 3c-d); therefore we do not conduct further interpretations of these particular deformations. In contrast, the third relative warp (11% of explained variance) shows a considerable deepening of the body, mostly in the abdomen section, in coastal populations

(Fig. 3e), whereas inland individuals appear to exhibit larger and narrower bodies (Fig. 3f).

## Discussion

### Habitat predicts superfetation and life history divergence

Fish from inland locations have higher levels of superfetation than those from coastal environments. Such variation in superfetation is consistent with previous reports of variation in this trait in other poeciliid fish species (Stearns 1978, Travis et al. 1987, Downhower et al. 2002). What is most notable about our findings is that variation in *P. turrubarensis* was related to differences in the stream gradient between habitats. Populations from inland environments, where water flow is on average faster than coastal environments, produce a higher number of broods (i.e. higher superfetation) than their coastal counterparts. This evidence is consistent with the hypothesis that stream velocity has been a selective factor for the origin and

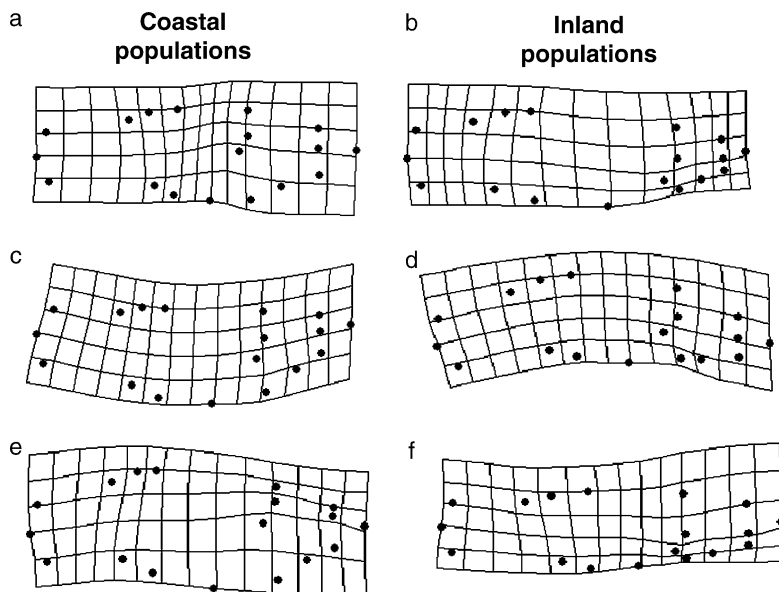


Fig. 3. Deformation grids depicting shape deviations from the overall consensus shape of individuals from coastal (left column) and inland (right column) populations. Panels (a) and (b) represent differences in shape based on scores on the first relative warp; (c) and (d) represent differences in shape based on scores on the second relative warp; and (e) and (f) represent differences in shape based on scores on the third relative warp. Together, these three relative warps account for the 48% of the variance in the data set. Deformations are 2x-scaled for visualization purposes. Black dots depict the 18 scored anatomical landmarks.

maintenance of variation in the degree of superfetation, presumably through the demands imposed by moving water on the morphology of individuals (below; Thibault and Schultz 1978, Reznick and Miles 1989).

Beyond superfetation, our study reveals several additional patterns of life history divergence between inland and coastal habitat types. For example, *P. turrubarensis* from inland sites actually have a higher overall level of reproductive investment than populations from coastal habitats. This greater reproductive investment is due completely to an increase in the number of broods per female. In other words, reproductive investment per brood does not differ between habitat types, only the number of broods (and by definition the number of offspring) per female does. Yet even at inland sites, reproductive allocation (as measured by wet brood mass) is lower as a result of increased superfetation than would be expected without superfetation. In other words, superfetation allows an increase in offspring production without the associated cost of increased brood mass at any given time. Finally, our results show a classic tradeoff between number and size of offspring – inland populations have more embryos per brood than coastal populations, but these embryos are significantly smaller. What accounts for these broader patterns of life history divergence remains unclear. A closer look examining patterns of mortality and possible differences in density or resource availability could provide

some insight into the causes of these observed life history differences.

Previous work suggested that *P. turrubarensis* showed no post-fertilization maternal provisioning to the embryos (i.e. was lecithotrophic; Reznick et al. 2002). Here we show that *P. turrubarensis* exhibits moderate nutrient transfer from the mother to developing young; dry mass of the embryo was approximately maintained during development (i.e. matrotrophy indexes near 1) for each of the populations we examined here. This implies some post-fertilization provisioning. Contrasting findings between this work and Reznick and colleagues (2002) suggests that a facultative shift between lecithotrophy and moderate matrotrophy in *P. turrubarensis* could occur within this species, and also that the observed variation might represent genetic differences among populations. Both cases are interesting possibilities whose existence and causes remain to be tested.

With few exceptions (Thibault and Schultz 1978, Reznick et al. 1996b, Lima 2005), superfetation has been associated with at least a certain amount of post-fertilization maternal provisioning to the embryos (Reznick and Miles 1989, Trexler and DeAngelis 2003), suggesting a functional relationship between these two modes of reproduction. Therefore, our intuitive expectation was to observe higher matrotrophy indexes in inland sites, where we found higher superfetation. However, estimated matrotrophy indexes were

not significantly different between coastal and inland populations, which indicated that the increment in the number of broods produced by upstream fish is not mediated by a higher maternal provisioning of nutrients to the embryos during development.

### Habitat predicts body shape

We found a striking difference in individual morphology between coastal and inland populations. These differences were associated with variation in the number of broods per female and between habitat types. In general, fishes from coastal sites are shorter and more robust than those in inland sites. The benefit of a streamlined morphology in high velocity (inland) environments could explain this pattern (Thibault and Schultz 1978, Reznick and Miles 1989). A shift toward a more streamlined phenotype is expected to enhance swimming performance in fishes (Lighthill 1975, Blake 1983, 2004, Webb 1984, Videler 1993, Vogel 1994, Boily and Magnan 2002) and has been documented in other neotropical fishes in flowing environments when compared to their still-water counterparts (Langerhans et al. 2003). Yet, streamlined bodies can also impose constraints on the capacity to carry an increased number of broods, which might seem contradictory to our findings of higher superfetation in individuals from inland populations. Nevertheless, it is feasible that fishes with larger bodies (such as those observed in inland sites) compensate for a loss of volume in the abdominal region by accommodating the higher number of broods in a longitudinal manner. In our study, we note a reduction of the caudal peduncle relative to the total length in inland individuals, which augments the body space potentially useful for carrying embryos. Evidence exists for other poeciliids of ovoid ovaries oriented along the main axis of the fish in species inhabiting moving water (Miller 1975, Thibault and Schultz 1978). The observed shift towards increased superfetation associated with a shift in body morphology in inland populations supports the 'morphological constraint' hypothesis for the origin and maintenance of variation in superfetation (Thibault and Schultz 1978, Reznick and Miles 1989).

### Alternative hypotheses and further research

The primary focus of this study was to test for an association between stream velocity and superfetation. However, we recognize that other environmental factors could contribute to the patterns observed here. For example, differences in food regimes have been considered as the base argument for another hypothesis that aims to explain the origin and variation of superfetation (Thibault and Schultz 1978, Travis et al. 1987, Reznick

and Miles 1989). The thrust of this competing hypothesis is that superfetation reduces peak demand of a brood on the female and thus, by partitioning the brood into subsets, the total energetic demand is spread over time. Therefore, superfetation should be favored in low resource environments. This model predicts that there should be an increase in superfetation when food resources are scarce. Although, we have no estimates of food availability between coastal and inland sites, at least one line of evidence suggests that food availability does not explain our results. Food restriction in guppies (*Poecilia reticulata*) and least killifish (*Heterandria formosa*) results in lower reproductive investments and lower fecundity, a finding exactly opposite of our observations for inland sites where superfetation is highest (Reznick et al. 1996b).

Differences in demographic environment can also affect life history variation. Travis et al. (1987) tested this idea in another superfetating, livebearing fish (*Heterandria formosa*) based on predictions proposed by Giesel (1976) and Burley (1980). Their results suggested that superfetation may have evolved in populations experiencing selection for increased rate of offspring production driven by low or uncertain adult survival relative to juvenile survival. In contrast, Downhower and Brown (1975) used a life table approach to argue that superfetation might be favored when adult female life expectancy is high and reduces the cost of peak reproduction. We do not have estimates of mortality rates for *P. turrubarensis*. However, our life history data show a pattern consistent with expectations if inland populations experience higher mortality rates than coastal populations; for example, inland populations produce more offspring per litter, produce litters more frequently, have smaller offspring, and have a higher overall reproductive investment. These differences correspond to what we see in two other livebearing fish species (guppies, *Poecilia reticulata*, and brachys, *Brachyrrhaphis rhabdophora*) where differences in mortality rates occur among populations (Reznick et al. 1996a, Johnson and Belk 2001); yet in both of those systems, mortality rates are highest in more coastal populations, opposite of what would be required to explain life history divergence in *P. turrubarensis*. Still, subsequent studies to gather mortality estimates across localities could be very useful in further exploring divergence in *P. turrubarensis* life histories, as they have been in other livebearing fish systems (Chapman and Kramer 1991, Reznick et al. 1996a, Johnson and Zúñiga-Vega, unpubl.).

Although other environmental factors could contribute to life history divergence in *P. turrubarensis*, our results are completely consistent with the 'morphological constraint' hypothesis. These findings suggest that stream velocity, through its effects on body shape, is a likely selective factor that could play a role in the

evolution of superfetation. However, the discovery that *P. turrubarensis* shows such marked reproductive variation offers important opportunities to test competing hypotheses about how superfetation evolves. Controlled experiments will provide the next step to understanding the effect that stream flow and morphological constraints have on the degree of superfetation in poeciliid fishes. With an experimental approach it will also be possible to explore the impact of potential interactions between stream flow and other environmental factors – such as food availability or mortality – providing further insight into ecological pressures that could help shape superfetation in natural systems. Finally, our results offer the first line of evidence that morphological constraints could be important in shaping superfetation in fishes. Whether or not morphological constraints also contribute to superfetation in species as diverse as plants and mammals remains an open question, but certainly one worth exploring.

*Acknowledgements* – We thank J. Rasmussen, C. Heizenrader, C. Allred, K. Elder, C. Jones and L. Scott for field assistance. M. Weitzmann, D. Melville, S. Warne, P. Scarborough, I. Dawson, C. Sayre, W. Alexander and S. Tilly helped with data collection in the lab. M. Belk provided comments on the morphometric analysis and R. B. Langerhans offered feedback on the relationship between water velocity and body shape. The Ministerio de Ambiente y Energía-Sistema Nacional de Áreas de Conservación from Costa Rica provided permits for field work. The Dept of Integrative Biology at Brigham Young Univ. and the Comisión Federal de Electricidad of México kindly provided postdoctoral fellowships to JJZV. J. Downhower provided comments that improved an earlier version of the manuscript.

## References

- Anonymous 2001. STATISTICA (data analysis software system), ver. 6.0. Statsoft, Inc. www.statsoft.com.
- Beck, C. W. and Beck, R. E. 2005. The effect of packing constraints on optimal investment in offspring. – *Evol. Ecol. Res.* 7: 1077–1088.
- Blake, R. W. 1983. Fish locomotion. – Cambridge Univ. Press.
- Blake, R. W. 2004. Fish functional design and swimming performance. – *J. Fish. Biol.* 65: 1193–1222.
- Boily, P. and Magnan, P. 2002. Relationship between individual variation in morphological characters and swimming costs in brook charr (*Salvelinus fontinalis*) and yellow perch (*Perca flavescens*). – *J. Exp. Biol.* 205: 1031–1036.
- Burley, N. 1980. Clutch overlap and clutch size: alternative and complementary reproductive tactics. – *Am. Nat.* 115: 223–246.
- Bussing, W. A. 1987. Peces de las aguas continentales de Costa Rica. – Editorial de la Univ. de Costa Rica.
- Cabrera-Peña, J. and Solano-López, Y. 1995. Fertilidad y fecundidad en *Poeciliopsis turrubarensis* (Pisces: Poeciliidae). – *Rev. Biol. Trop.* 43: 317–320.
- Chapman, L. J. and Kramer, D. L. 1991. The consequences of flooding for the dispersal and fate of poeciliid fish in an intermittent tropical stream. – *Oecologia* 87: 299–306.
- Downhower, J. F. and Brown, L. 1975. Superfetation in fishes and the cost of reproduction. – *Nature* 256: 345.
- Downhower, J. F. et al. 2002. Litter overlap in *Gambusia hubbsi*: superfetation revisited. – *Environ. Biol. Fish.* 65: 423–430.
- Ghalambor, C. K. et al. 2004. Constraints on adaptive evolution: the functional tradeoff between reproduction and fast-start swimming performance in the Trinidadian guppy (*Poecilia reticulata*). – *Am. Nat.* 164: 38–50.
- Giesel, J. T. 1976. Reproductive strategies as adaptations to life in temporally heterogeneous environments. – *Annu. Rev. Ecol. Syst.* 7: 57–80.
- Glazier, D. S. 2000. Smaller amphipod mothers show stronger tradeoffs between offspring size and number. – *Ecol. Lett.* 3: 142–149.
- Haynes, J. D. 1995. Standardized classification of poeciliid development for life-history studies. – *Copeia* 1995: 147–154.
- Johnson, J. B. and Belk, M. C. 2001. Predation environment predicts divergent life-history phenotypes among populations of the livebearing fish *Brachyrhaphis rhabdophora*. – *Oecologia* 126: 142–149.
- Johnson, J. B. et al. 2004. Neglected taxonomy of rare desert fishes: congruent evidence for two species of leatherside chub. – *Syst. Biol.* 53: 841–855.
- Kennedy, H. 1978. Systematics and pollination of the “closed-flowered” species of *Calathea* (Marantaceae). – *Univ. Calif. Publ. Bot.* 71: 1–90.
- Langerhans, R. B. et al. 2003. Habitat-associated morphological divergence in two Neotropical fish species. – *Biol. J. Linn. Soc.* 80: 689–698.
- Lighthill, M. J. 1975. Mathematical biofluidynamics. – Soc. Appl. Industrial Math., Philadelphia, PA.
- Lima, N. R. W. 2005. Variations on maternal-embryonic relationship in two natural and six laboratory made hybrids of *Poeciliopsis monacha-lucida* (Pisces, Cyprinodontiformes). – *Braz. Arch. Biol. Techn.* 48: 73–79.
- Meisner, A. D. and Burns, J. R. 1997. Viviparity in the halfbeak genera *Dermogenys* and *Nomorhamphus* (Teleostei: Hemiramphidae). – *J. Morphol.* 234: 295–317.
- Miller, R. R. 1975. Five new species of Mexican poeciliid fishes of the genera *Poecilia*, *Gambusia*, and *Poeciliopsis*. – *Ocass. Pap. Mus. Zool. Univ. Mich.* 672: 1–44.
- Reznick, D. N. 1989. Life history evolution in guppies. 2. Repeatability of field observation and the effects of season on life histories. – *Evolution* 43: 1285–1297.
- Reznick, D. N. and Miles, D. B. 1989. Review of life history patterns in poeciliid fishes. – In: Meffe, G. K. and Snelson, F. F., Jr. (eds), *Ecology and evolution of livebearing fishes (Poeciliidae)*. Prentice Hall, pp. 125–148.
- Reznick, D. et al. 1993. Life history of *Brachyrhaphis rhabdophora* (Pisces: Poeciliidae). – *Copeia* 1993: 103–111.

- Reznick, D. N. et al. 1996a. Life-history evolution in guppies (*Poecilia reticulata*). 6. Differential mortality as a mechanism for natural selection. – *Evolution* 50: 1651–1660.
- Reznick, D. et al. 1996b. Maternal effects on offspring quality in poeciliid fishes. – *Am. Zool.* 36: 147–156.
- Reznick, D. N. et al. 2002. Independent origins and rapid evolution of the placenta in the fish genus *Poeciliopsis*. – *Science* 298: 1018–1020.
- Roff, D. A. 1992. The evolution of life histories: theory and analysis. – Chapman and Hall.
- Roff, D. A. 2002. Life history evolution. – Sinauer Ass.
- Rohlf, F. J. 2002. TPSRELW. – Dept Ecol. Evol., State Univ. of New York.
- Scrimshaw, N. S. 1944. Superfetation in poeciliid fishes. – *Copeia* 1944: 180–183.
- Stearns, S. C. 1978. Interpopulational differences in reproductive traits of *Neobeterandria tridentiger* (Pisces: Poeciliidae) in Panamá. – *Copeia* 1978: 188–190.
- Stearns, S. C. 1992. The evolution of life histories. – Oxford Univ. Press.
- Thibault, R. E. and Schultz, R. J. 1978. Reproductive adaptations among viviparous fishes (Cyprinodontiformes: Poeciliidae). – *Evolution* 32: 320–333.
- Travis, J. et al. 1987. Testing theories of clutch overlap with the reproductive ecology of *Heterandria formosa*. – *Ecology* 68: 611–623.
- Trexler, J. C. and DeAngelis, D. L. 2003. Resource allocation in offspring provisioning: an evaluation of the conditions favoring the evolution of matrotrophy. – *Am. Nat.* 162: 574–585.
- Turner, C. L. 1937. Reproductive cycles and superfetation in poeciliid fishes. – *Biol. Bull.* 72: 145–164.
- Turner, C. L. 1940. Superfetation in viviparous cyprinodont fishes. – *Copeia* 1940: 88–91.
- Videler, J. J. 1993. Fish swimming. – Chapman and Hall.
- Vogel, S. 1994. Life in moving fluids. – Princeton Univ. Press.
- Webb, P. W. 1984. Body form, locomotion, and foraging in aquatic vertebrates. – *Am. Zool.* 24: 107–120.
- Wourms, J. P. et al. 1988. The maternal embryonic relationship in viviparous fishes. – In: Hoar, W. S. and Randall, D. J. (eds), *Fish physiology*, vol. 11B. Academic Press, pp. 1–134.
- Yamaguchi, N. et al. 2004. Multiple paternity and reproductive tactics of free-ranging American minks, *Mustela vison*. – *J. Mammal.* 85: 432–439.
- Yamaguchi, N. et al. 2006. Female receptivity, embryonic diapause, and superfetation in the European badger (*Meles meles*): implications for the reproductive tactics of males and females. – *Q. Rev. Biol.* 81: 33–48.
- Zelditch, M. L. et al. 2004. Geometric morphometrics for biologists: a primer. – Elsevier Academic Press.