

UC Riverside

UC Riverside Electronic Theses and Dissertations

Title

Ecomorphology of Body Shape and Life History in two Poeciliids (Gambusia spp. and Poecilia reticulata)

Permalink

<https://escholarship.org/uc/item/8fh4710b>

Author

Torres-Mejia, Rafael Mauricio

Publication Date

2011

Peer reviewed|Thesis/dissertation

UNIVERSITY OF CALIFORNIA
RIVERSIDE

Ecomorphology of Body Shape and Life History in Females of the Genus
Gambusia (Poeciliidae) and in Guppies (*Poecilia reticulata*, Poeciliidae)

A Dissertation submitted in partial satisfaction
of the requirements for the degree of

Doctor of Philosophy

in

Evolution, Ecology, and Organismal Biology

by

Rafael Mauricio Torres-Mejia

December 2012

Dissertation Committee:

Dr. David Reznick, Chairperson

Dr. Kurt Anderson

Dr. Theodore Garland, Jr.

Dr. Derek Roff

Copyright by
Rafael Mauricio Torres-Mejia
2012

The Dissertation of Rafael Mauricio Torres-Mejia is approved:

Committee Chairperson

University of California, Riverside

Acknowledgements

I have everything to thank to my advisor David Reznick. I could have never imagined, not even in my wildest optimistic dreams, that my PhD mentor was going to be such a smart, generous, patient, supportive, and likable person. Tropical fevers are great stimulant for ideas: one of them gave Alfred Russell Wallace the notion of evolution by natural selection, and in my case I got from a dengue fever the impulse to apply to work with David. Both are brilliant ideas that have brough loads of amusement to my life.

I enjoyed the benefits of a remarkable dissertation committee. Brian Langerhans was a commitee member by all means. He was very kind to share with me many interesting ideas about the *Gambusia* system, geometric morphometrics, evolutionary biology, and, why not saying it, gonopodia.

I was very fortunate to interact with Ted Garland. His class on comparative methods was not only truly enlighting but also masterfully imparted. The many times I interrupted his schedule to talk about the dissertation resulted in memorable and rewarding events.

Derek Roff was specially crucial to my career. Derek was part of every single committee during my PhD, and in all of them he gave insightful advice and contributed with very sharp and constructive perspectives.

Kurt Anderson made important contributions to the dissertation with his knowledge on the ecology of flow regimes. His knowledge on this unique topic

was of great support when I attempted to extend my project in that novel direction.

I am also thankful to other faculty and staff members at UCR. Douglas Altshuler made the theory and methods of biomechanics smooth and delightful to grasp. Moreover, his charming conversations about the whereabouts of science account for some of the best moments as a graduate student. Learning about multivariate statistics and ecological theory from John Rotemberry was literally as cool as if I had taken those classes from the Big Lebowski, perhaps more. Working with John Gatesy designing a laboratory manual about Systematics for introductory biology was a very rewarding and edifying experience. The enthusiasm for the natural world and the eagerness to know of Daphne Fairbairn were truly contagious, I want to be that kind of naturalist when I grow up. The inspiring teaching endeavor of Tracy Kahn made a lasting impression. Shardai Williams, Mellisa Gomez, Perla Fabelo, Michael Fugate, John Oross, and the lab prep crew, Esther Valdez, Jon Allen, Mi Kyong Kim, and Xinxia Li, highly improved the quality of life at UCR.

There are many postdocs and graduate students at UCR that I should acknowledge. Andrés López-Sepulcre simply made my life viable by teaching me about R. His friendship, iteratively reminded through extended coffee breaks, made me a better Hispanoamerican. Jeff Arendt was a great mentor in many ways, and the best person to cross the continent in a road trip. I was lucky to be in a superfetitious and highly matrotrophic but not adelphotrophic lab. I learned a

lot about science and life from interacting with Marcelo Pires, Mandy Banet, Matt Walsh, Martin Turcotte, Sonya Auer, Ron Bassar, Swanne Gordon, Andrew Furness, and Keenan Morrison. Special thanks to Melody Clark for her hospitality and for taking care of us all. Ron and Sonya were also very kind to be my landlords when I needed it the most. Andrew played specially supported me in the last phase of the process. It was never clear if Chris Oufiero was a lab member in disguise; in any case he was always willing to help with his skills on comparative methods and biomechanics of fish swimming. I am very glad I met Gabriel Gardner, Brooke Keeney, Eric Kolb, Kyle Van Dollah, Terry Orr, Breanna Harris, Erin Conlisk, Sonia Ortiz, Lijin Zeng, Brian Grey, Byron Weckworth, Laramy Enders, Sarah French, Bart Pollux, Vincent Careau, and Christine Pinard. Special thanks to the last two for helping me smuggling undocumented immigrants to Canada.

In the laboratory, Yuridia Reynoso was the mastermind behind the coordination of undergrads, they made this project possible: Peter Ajoku, Ryan Bedi, Sebastián Benavides, Michelle Bui, Elizabeth Geledzhyan, Sunny Lee, Kristine Mantaring, Atiqa Mateen, Brittany Morello, Maryam Mushtaq, Samantha Natividad, Vyvian Ngo, Thanh Nguyen, Merillu Q., Victoria Senechal, Manuela Serrano, Dorothy Skates, Kieu-Chinh Tran, Vy Tran, Peng Wang, and Jerry Yeh. Special thanks to Ryan, Michelle, Vyvian, Jerry, Kieu, and Sebastian for their dedication.

The field work was the most exciting part of this research, and the following people highly contributed to make it happen: Rafael Oyaga, Jaime Viana, Andrés López-Sepulcre, Swanne Gordon, Justa Heinen, Pierre de Villemeruil, Carl & Kelly Fitzjames, David Owens, Katherine Gleason, Alisha Shah, Julián Torres Dowdall, María Hernández, Maryen Torres, Bret Robinson, Karen Sullam, Elizabeth Hassell, Nicole Bredford, Ikyrra Francois, Elsa Ordway, Mike Marshall, Sarah Fitzpatrick, Dale Broder, Troy Simon, and Keeley McNeill.

Other people outside of UCR also contributed to this project. The interactions with Matt McHenry, John Sabo, David Lytle, Helen Rodd, and Steve Thomas were instrumental to development of ideas. Steve was also very kind to let me use his flow meter equipment. Douglas Nelson (UMMZ), Dean Hendrickson (TNHC), Jessica Rosales (TNHC), Mark Sabaj (ANSP), John Lundberg (ANSP), Lynne Parenti (USNM), Jeff Williams (USNM), Scott Schaefer (AMNH), Rob Robins (UF), Tabitha Vigliotti (UF), Heather Prestidge (TCWC), Martha P. Ramírez-Pinilla (UIST), and Kathleen Quigley (NCSU) kindly facilitated loan of specimens.

Doing a PhD while raising a family is a particular experience. Fortunately we had wonderful friends that not only were there when we needed but also made everything quite fun: Magdalena, Higinio, Yolis, Marisela, César, Poncho, El Danny, Bárbara, Edmundo, Ana María, Oscar, Gloria, Henry, Kelly, Elo, Javier, Katy, Pablo, Iván Darío Barrero, Marcela Pierce, Perla, Juanita at the CDC, Javedo and Ángela, Mario and Silvia, Sylvia and Doug, and of course Ale,

Quicazán, Edgar Chávez, Jelena, Andrelo, Swancita, and Raymundo. Gracias y thank you.

I would not be anywhere without the inspiration and encouragement from my family. The efforts of Pablo and Luzmi pushed me all the way to here. Tía Myriam, Monica, Mayen, and Myyo, each one in a different way helped me to finish this project. My in-law family were also very important in this whole process, thanks Fabio, Nora, Lucas, Sergio, Iván, tía Elsa, Gabriel, tía Irma, Fanny, Mariana and Manuela. Finally, thanks to Pame for holding the foundations that supported the whole structure while I was in this semi-present state. And thanks to Violeta for being the nearest sun.

This work was funded by UCR (DD-EEOB, DG-EEOB, and GD-EEOB fellowships awarded to RMTM) and NSF (DEB 0416085 to D. N. Reznick and DEB-0623632 to D. N. Reznick and others).

This is dedicated to Rosa and José, who showed me the beauty of nature and
were always part of it

ABSTRACT OF THE DISSERTATION

Ecomorphology of Body Shape and Life History in Females of the Genus *Gambusia* (Poeciliidae) and in Guppies (*Poecilia reticulata*, Poeciliidae)

by

Rafael Mauricio Torres-Mejia

Doctor in Philosophy, Graduate Program in Evolution, Ecology, and Organismal
Biology

University of California, Riverside, December 2012

Dr. David N. Reznick, Chairperson

Multiple, potentially interacting features of the environment and the phenotype shape the way organisms evolve through adaptation. Ecomorphology aims to understand such intricate relationships between and within environmental and phenotypic variables. In this dissertation I made an integrative analysis of the combined effects of current velocity and predation on the evolution of body shape and life history of fishes.

In the first chapter I made an interspecific analysis of body shape in *Gambusia* females. As predicted, they showed larger caudal areas in high-predation sites. In contrast, flow had a small influence on shape. Pregnancy reduced the magnitude of phenotypic divergence between females from contrasting predation and flow regimes. These results indicate that adaptation to predation, and to a lesser extent flow, is key to body shape evolution in females of the genus *Gambusia*.

In the second chapter I analyzed the macroevolutionary pattern of life history in female *Gambusia* and its relationship with predation, flow, and latitude. *Gambusia* females showed a large range of variation in reproductive allocation, number of embryos, and size of embryos. One species of *Gambusia* showed incipient levels of maternal provisioning. Female body size was the best predictor of interspecific life history variation. Life history was not related with flow, or latitude, but females in high predation sites increase in ovary size at greater rates as they grow than females in low predation sites.

In the third chapter I explored more in depth the relationship between flow regime and body shape, comparing populations of Trinidadian guppies from sites with contrasting hydrologies. We tested the correlation between guppy shape, geomorphological features correlated with flow regime (watershed area, relief, and shape), and other environmental characteristics potentially interacting with guppy shape (pool volume, canopy cover, flow velocity, and the abundance of prawns and *Rivulus hartii*). We observed a correlation between body shape and geomorphological features of the watersheds. However, these results varied between sexes. We found gradients of prawn and *Rivulus* abundances that explained some of the body shape variation. Additionally, we found that pregnancy dissipated the correlations between environmental variables and shape.

Table of Contents

Introduction.....	1
Literature Cited.....	9
1. Body shape evolution in female <i>Gambusia</i> (Poeciliidae): a phylogenetic approach with a comparison of morphometric methods.....	16
Abstract.....	16
Introduction.....	18
Methods.....	22
Results.....	35
Discussion.....	38
Literature Cited.....	49
Appendices.....	72
2. Independence of life histories to gradients of predation, flow, and latitude in <i>Gambusia</i> (Poeciliidae).....	127
Abstract.....	127
Introduction.....	129
Methods.....	134
Results.....	142
Discussion.....	144
Literature Cited.....	152
Appendices.....	176

3. Adaptation to disturbances: Effect of flow regime on body shape and life history of Trinidadian guppies.....	184
Abstract.....	184
Introduction.....	186
Methods.....	190
Results.....	199
Discussion.....	201
Literature Cited.....	208
Appendices.....	226
Concluding Remarks.....	231
Literature Cited.....	234

List of Tables

Table 1.1. Overview of collections analyzed.....	62
Table 1.2. MANCOVAs for the three configuration sets (shapes early in pregnancy, late in pregnancy, and the pregnancy effect).....	63
Table 1.3. Regressions showing best-supported model and average model for each dependent variable.....	64
Table 2.1. Environmental characteristics and average life histories of <i>Gambusia</i> samples.....	167
Table 2.2. Principal Component Analysis of log-transformed life history traits...	169
Table 2.3. Phylogenetic signal (K) of dependent and independent variables....	170
Table 2.4. Regressions, showing best-supported model and average model for each trait.....	171
Table 3.1. Environmental data from collections sites.....	219
Table 3.2. Bivariate correlations between predictor variables.....	220
Table 3.3. MANCOVAs for the configurations of females early in pregnancy....	221
Table 3.4. MANCOVA for the configurations of males.....	222

List of Figures

Figure 1.1 Map with collection localities and <i>Gambusia</i> phylogeny superimposed.....	67
Figure 1.2. (A) Digital fish reconstruction as a composite of 200 elliptical cylinders, used for calculating streamlining index and surface area of each fish. (B) Landmarks (circles) and semilandmarks (squares) digitized for geometric-morphometric analyses.....	68
Figure 1.3. Shape variation estimated with MANCOVAs.....	69
Figure 1.4. Left panel: Predicted values obtained from OLS models showing the predation effect on streamlining index (SI) of females early and late in pregnancy. Right panel: Lateral outlines of fish with SI values at the extremes of the SI range depicted in the left panel.....	71
Figure 2.1. Representation of the Principal Component 2, showing the relationship between the two variables with loadings > 0.32 in that axis.....	173
Figure 2.2. Association between mean values of log Standard Length and mean values of life history traits.....	174
Figure 2.3. Relationship between predation and the allometric coefficient of ovary dry weight for each collection.....	175
Figure 3.1. Sampling localities.....	223
Figure 3.2. Shape variation estimated with MANCOVAs.....	224
Figure 3.3. Body size and shape variation estimated with regressions.....	225

Introduction

Multiple, potentially interacting features of the environment and the phenotype shape the way organisms evolve through adaptation (Reznick and Travis 1996). Ecomorphology aims to understand such intricate relationships between and within environmental and phenotypic variables (Arnold 1983; Garland and Losos 1994). Even though a multidimensional understanding of adaptation has been advocated (Ricklefs and Miles 1994), such integrative approaches are still rare (Walker 1997). Here we embrace complexity by analyzing how multiple correlated phenotypic characteristics are determined by multiple environmental factors.

A general concept in evolutionary biology is that adaptation within species can explain patterns of adaptive variation among higher taxa (Darwin 1872: 49; Watson et al. 1936: 58; Charlesworth et al. 1982; Arnold et al. 2001). However, an alternative view is that divergence within species cannot always explain divergence at the macroevolutionary scale (Goldschmidt 1940; Van Valen 1974; Stanley 1979; Eldredge and Cracraft 1980) because the direction of phenotypic divergence can be modified by other evolutionary processes (e.g. random genetic drift). A useful approach to test the evolutionary continuum among scales intra- and interspecific scales is to produce models of divergence from microevolutionary patterns to predict patterns at the macroevolutionary level (Arnold et al. 2001; Losos and Ricklefs 2009). In the first two chapters of the

dissertation I used this strategy to explain the interspecific patterns of body shape and life history in female *Gambusia* in response to predation and flow, testing predictions derived from models that were developed to assess microevolutionary processes.

Predation is a crucial biotic factor in nature. Organisms have evolved diverse strategies to avoid or escape from predators, from behavioral (e.g. activity patterns, habitat preferences, faking death, vigilance), to morphological (e.g. horns and spikes, camouflage, aposematic colorations), to biochemical (e.g. chemical defense), and the list continues (Langerhans 2006). One mechanism to escape from predators is to move away from them. To do so organisms may evolve shapes that improve an escape response (Langerhans 2009). Biomechanical theory states that in fishes the hydrodynamic efficiency is proportional to streamlining of anterior area of body and to depth of caudal region (Walker 1997). As predicted by biomechanical theory, fishes living in high-predation environments have an increased caudal area.

Life history theory predicts that high mortality risk in adults (e.g. due to predation) favors increased reproductive effort (Gadgil and Bossert 1970; Law 1979). Empirical studies have confirmed such predictions. In independent studies it has been observed that in habitats with high predation risk Poeciliid fishes have smaller embryos, higher fecundities, and mature younger (Reznick and Endler 1982; Reznick et al. 1996; Johnson and Belk 2001; Jennions and Telford 2002; Reznick et al. 2002). For example, females of fishes living in sites with predatory

fishes have higher reproductive allotments in *Poecilia reticulata* (Reznick and Endler 1982) and *Gambusia hubbsi* (Downhower et al. 2000).

The dynamics of medium (i.e. air, water) may exert a powerful effect on phenotypes. Such effects are apparent in terrestrial habitats, where strong winds uproot trees (Ennos 1999) and restrict bird migration (Erni et al. 2005). However they are more evident in aquatic systems, where up to 95% of fish in a stream may be washed-out by flash floods (Chapman and Kramer 1991). Fishes evolve characteristics morphologies to avoid being flushed-out by strong currents. As expected by biomechanical theory, high water velocity is related with larger caudal areas. It has been hypothesized that high body depth is related with slow water velocities (Gatz 1979), but in general the effect of water velocity on streamlining, abdomen distention, or head size is not clear.

The relationship between water velocity and life history of fishes has received little attention. It is unknown how the mortality schedule is influenced by water velocity. There is some evidence that swimming performance is lower at young ages (Humphries et al. 1999), which may decrease the chances of surviving after floods. Therefore even exploratory information about life history variation in response to current is needed.

Body shape and life history are interrelated. Data from 23 lizard species indicated that morphology was a good predictor of relative clutch mass (Vitt and Congdon 1978). Such morphology was related with foraging (“sit and wait” vs “wide foraging”) and with escape responses. More streamlined lizards (the wide

foragers) have relatively smaller clutch masses, what was explained as the effect of a limited abdomen volume to invest in reproductive functions. Further studies have supported such hypothesis (Vitt 1981; Griffith 1994; Forsman and Shine 1995; Pizzatto et al. 2007), but there is some disagreement (Ballinger et al. 2000). Conversely, reproduction may impair performance, likely through a modification of body shape. Theoretical and empirical studies have shown that body shape determines swimming performance (Domenici 2003; Langerhans et al. 2004). To have an extended abdomen may increase drag, and therefore reduce swimming performance (Plaut 2002). Ghalambor et al (2004) found that embryos of livebearing fish (*Poecilia reticulata*) increase in volume as development progressed. At the same time the locomotor performance of mothers declined. The rate of decline was faster in guppies from high predation localities. The high predation guppies had higher rates of acceleration when they were not gravid or when the young were early in development, but they lost this performance advantage when the young were approaching the end of development. The carrying of embryos or eggs has been proposed to reduce locomotor performance abilities also in lizards (Husak 2006), salamanders (Finkler et al. 2003), and snakes (Shine 2003).

Separate analyses of body shape, and life history in response to water velocity and predation would forfeit the opportunity to understand how all of these factors might interact in shaping the phenotype of fishes. For that reason a multidimensional analysis is desirable. However, it is hard to find a biological

system that offers clear gradients of the environmental variables in question (predation and current). Even in that case, it is preferable that variation through such gradients were due to multiple rather than unique evolutionary invasions to alternative environments, what permits formal comparative analyses. In this dissertation I will show why Poeciliids of the genus *Gambusia* and guppies (*Poecilia reticulata*) are such desirable systems.

The *Gambusia* system: an opportunity to study the the effect of predation and flow on body shape and life history at the interspecific level

The more than 44 current species that compose the genus *Gambusia* are naturally distributed from North America to Colombia, and in the Caribbean islands (Rosen and Bailey 1963; Lucinda 2003). Their habitats range from mountain streams to brackish waters. Populations of several species of *Gambusia* have colonized multiple times, naturally or by deliberate introduction, a diversity of habitats with a large range of predation and water velocity regimes. On the other hand, some species have a characteristic habitat, which can be readily classified as low- or high-predation or water current regimes. The current phylogenetic hypotheses for *Gambusia* (B. Langerhans, unpub.) show that such invasion of different habitats may have been independent. Therefore *Gambusia* could be viewed as a natural evolutionary experiment with replicates and performed at intra- and interspecific levels.

Gambusia has a large variation in body shape and life history. Body shapes vary from blunt- to stylized looking species. Even among populations, there are patterns of shape variation clear the naked eye, like the two morphs of *G. yucatanana* reported for low- and high-current habitats (Greenfield et al. 1983). The sparse life history data for species of *Gambusia* show a two-fold variation in size at maturity, a four-fold variation in embryo size, a ten-fold variation in reproductive allocation, and an astonishing 50-fold variation in fecundity within the genus (Krumholz 1963; Reznick and Miles 1989; Daniels and Felley 1992; Downhower et al. 2000; Abney and Rakocinski 2004; Pyke 2005). This range of variation is outstanding for a genus with only a two-fold range of body size.

In the first chapter of my dissertation I perform an interspecific analysis in females of 22 species of *Gambusia*, while taking into account the effect of phylogeny, pregnancy, and size. I use several commonly methods to quantify shape and discuss the differences among them. I show that female *Gambusia* have larger caudal areas in high-predation sites, in agreement to biomechanical theory. In contrast, flow regime is not related to body shape, against theoretical predictions, although an interaction between flow and predation was detected. We also observe that pregnancy reduced the magnitude of phenotypic divergence between females from contrasting predation and flow regimes. Most models showed high levels of phylogenetic signal. These results indicate that adaptation to predation, and to a lesser extent flow, is key to body shape

evolution in the genus *Gambusia*. Additionally, the importance of phylogenetic history and the role of pregnancy in explaining shape are discussed.

In the second chapter I analyze the effect of predation, flow, and latitude in life history of *Gambusia*. Surprisingly, despite there is a large range of life history variation in *Gambusia*, and predation and flow explain some of the body shape variation, life history was not related with predation or flow. We explored the correlation of life history with latitude, another factor associated with reproduction, and life history still remained unexplained.

Guppies as an ideal system to study the the effect flow regime on body shape and life history at the intraspecific level

In the third chapter of the dissertation I focus on flow, analyzing flow regime as a disturbance. Disturbances are ubiquitous phenomena (e.g. fires, floods, earthquakes) well recognized in ecological literature as agents that can disrupt ecosystem, community, or population structure. Even though disturbances can be seen as selective factors characterized by intensity, frequency, timing, and predictability (Lytle and Poff 2004), the adaptive evolution of organisms in response to disturbances has rarely been addressed (Lytle 2001). Flash floods in mountain streams are ideal phenomena to understand the evolutionary implications of disturbances. Flash floods are short-lived but severe (Swanson et al. 1998), causing high mortalities to aquatic fauna. Moreover, it is possible to

quantify and analyze the hydrological regime of mountain rivers using tools developed by engineers and geologists (Gordon et al. 2004). By contrasting the hydrology of mountain rivers against the natural history of their biota one can observe the evolutionary implications of these disturbances.

I study the evolutionary consequences of flash floods on body shape and life history of Trinidadian guppies. Biomechanical theory predicts that fishes may reduce costly swimming in turbulent flows, like those created by floods (Liao 2007), by optimizing unsteady (non-constant velocity) swimming useful for acceleration. Since guppies use their tails for propulsion during unsteady swimming, it is expected that guppies from flood-prone sites will have larger caudal areas. Life history theory predicts that a differential mortality among age classes would cause a change in optimal reproductive effort. Empirical studies have confirmed such predictions regarding predation. It is known that flash floods cause higher mortality in smaller fish (Chapman and Kramer 1991). Therefore guppies living under contrasting flow regimes should vary in life history traits, such as reproductive allocation. That was exactly what we found, although the variation was in the opposite direction, perhaps because of density dependence. We conducted a comparative survey across natural populations with contrasting flow regimes in order to determine if the hydrological variation was related with the phenotypic variation of interest.

Literature Cited

- Abney, M., and C. Rakocinski. 2004. Life-history variation in Caribbean gambusia, *Gambusia puncticulata puncticulata* (Poeciliidae) from the Cayman Islands, British West Indies. *Environ. Biol. Fishes* 70:67-79.
- Arnold, S. J. 1983. Morphology, performance and fitness. *Am. Zool.* 23:347-361.
- Arnold, S. J., M. E. Pfrender, and A. G. Jones. 2001. The adaptive landscape as a conceptual bridge between micro- and macroevolution. *Genetica* 112-113:9-32.
- Ballinger, R. E., J. A. Lemos-Espinal, and G. R. Smith. 2000. Reproduction in females of three species of crevice-dwelling lizards (genus *Xenosaurus*) from Mexico. *Studies on Neotropical Fauna and Environment* 35:179-183.
- Chapman, L. J., and D. L. Kramer. 1991. The consequences of flooding for the dispersal and fate of poeciliid fish in an intermittent tropical stream. *Oecologia* 87:299-306.
- Charlesworth, B., R. Lande, and M. Slatkin. 1982. A Neo-Darwinian commentary on macroevolution. *Evolution* 36:474-498.
- Daniels, G. L., and J. D. Felley. 1992. Life history and foods of *Gambusia affinis* in two waterways of southwestern Louisiana. *The Southwestern Naturalist* 37:157-165.
- Darwin, C. 1872. *On the origin of species by means of natural selection*. Murray, London, UK.

- Domenici, P. 2003. Habitat, body design and the swimming performance of fish. Pp. 137–160 in V. L. Bels, J. P. Gasc, and A. Casinos, eds. Vertebrate Biomechanics and Evolution. BIOS Scientific Publishers Ltd, Oxford.
- Downhower, J. F., L. P. Brown, and M. L. Matsui. 2000. Life history variation in female *Gambusia hubbsi*. Environ. Biol. Fishes 59:415-428.
- Eldredge, N., and J. Cracraft. 1980. Phylogenetic Patterns and the Evolutionary Process. Columbia University Press, New York, NY.
- Ennos, A. R. 1999. The aerodynamics and hydrodynamics of plants. J. Exp. Biol. 202:3281-3284.
- Erni, B., F. Liechti, and B. Bruderer. 2005. The role of wind in passerine autumn migration between Europe and Africa. Behav. Ecol. 16:732-740.
- Finkler, M. S., M. T. Sugalski, D. L. Claussen, and S. J. Beaupre. 2003. Sex-related differences in metabolic rate and locomotor performance in breeding spotted salamanders (*Ambystoma maculatum*). Copeia 2003:887-893.
- Forsman, A., and R. Shine. 1995. Parallel geographic variation in body shape and reproductive life history within the Australian scincid lizard *Lampropholis delicata*. Funct. Ecol. 9:818-828.
- Gadgil, M., and W. H. Bossert. 1970. Life historical consequences of natural selection. The American Naturalist 104:1-24.
- Garland, T., Jr., and J. B. Losos. 1994. Ecological morphology of locomotor performance in squamate reptiles. Pp. 240-302 in P. C. Wainwright, and S.

- M. Reilly, eds. Ecological Morphology: Integrative Organismal Biology. Chicago University Press, Chicago.
- Gatz, A. J., Jr. 1979. Ecological morphology of freshwater stream fishes. Tulane Studies in Zoology and Botany 21:91-124.
- 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*). The American Naturalist 164:38-50.
- Goldschmidt, R. 1940. The material basis of evolution. Yale University Press, New Haven, CT.
- Gordon, N. D., T. A. McMahon, B. L. Finlayson, C. J. Gippel, and R. J. Nathan. 2004. Stream Hydrology: an introduction for ecologists. John Wiley & Sons, West Sussex, UK.
- Greenfield, D. W., T. A. Greenfield, and S. L. Brinton. 1983. Spatial and trophic interactions between *Gambusia sexradiata* and *Gambusia puncticulata yucatanana* (Pisces: Poeciliidae) in Belize, Central America. Copeia 1983:598-607.
- Griffith, H. 1994. Body elongation and decreased reproductive output within a restricted clade of lizards (Reptilia: Scincidae). J. Zool. 233:541-550.
- Humphries, P., A. J. King, and J. D. Koehn. 1999. Fish, flows and flood plains: Links between freshwater fishes and their environment in the Murray-Darling river system, Australia. Environ. Biol. Fishes 56:129-151.

- Husak, J. 2006. Do female collared lizards change field use of maximal sprint speed capacity when gravid? *Oecologia* 150:339-343.
- Jennions, M. D., and S. R. Telford. 2002. Life-history phenotypes in populations of *Brachyrhaphis episcopi* (Poeciliidae) with different predator communities. *Oecologia* 132:44-50.
- Johnson, J. B., and M. C. Belk. 2001. Predation environment predicts divergent life-history phenotypes among populations of the livebearing fish *Brachyrhaphis rhabdophora*. *Oecologia* 126:142-149.
- Krumholz, L. A. 1963. Relationships between fertility, sex ratio, and exposure to predation in populations of the mosquitofish *Gambusia manni* Hubbs at Bimini, Bahamas. *Internationale Revue der gesamten Hydrobiologie und Hydrographie* 48:201-256.
- Langerhans, R. B. 2006. Evolutionary consequences of predation: avoidance, escape, reproduction, and diversification in A. M. T. Elewa, ed. *Predation in organisms: a distinct phenomenon*. Springer-Verlag, Heidelberg, Germany.
- Langerhans, R. B. 2009. Morphology, performance, fitness: functional insight into a post-Pleistocene radiation of mosquitofish. *Biol. Lett.* 5:488-491.
- 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.

- Law, R. 1979. Optimal life histories under age-specific predation. *The American Naturalist* 114:399-417.
- Liao, J. C. 2007. A review of fish swimming mechanics and behaviour in altered flows. *Philosophical Transactions of the Royal Society B: Biological Sciences* 362:1973-1993.
- Losos, J. B., and R. E. Ricklefs. 2009. Adaptation and diversification on islands. *Nature* 457:830-836.
- Lucinda, P. H. F. 2003. Poeciliidae. Pp. 555-581 *in* R. E. Reis, S. O. Kullander, and C. J. Ferraris, eds. *Checklist of the Freshwater Fishes of South and Central America*. EDIPUCRS, Porto Alegre, Brasil.
- Lytle, D. A. 2001. Disturbance regimes and life-history evolution. *The American Naturalist* 157:525-536.
- Lytle, D. A., and N. L. Poff. 2004. Adaptation to natural flow regimes. *Trends Ecol. Evol.* 19:94-100.
- Pizzatto, L., S. M. Almeida-Santos, and R. Shine. 2007. Life-history adaptations to arboreality in snakes. *Ecology* 88:359-366.
- Plaut, I. 2002. Does pregnancy affect swimming performance of female Mosquitofish, *Gambusia affinis*? *Funct. Ecol.* 16:290-295.
- Pyke, G. 2005. A review of the biology of *Gambusia affinis* and *G. holbrooki*. *Rev. Fish Biol. Fish.* 15:339-365.

- Reznick, D., M. J. Bryant, and F. Bashey. 2002. r- and K-selection revisited: the role of population regulation in life-history evolution. *Ecology* 83:1509-1520.
- 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., and D. B. Miles. 1989. Poeciliid life history patterns. Pp. 373-377 *in* G. K. Meffe, and F. F. Snelson, eds. *Ecology and evolution of livebearing fishes (Poeciliidae)*. Prentice Hall, Englewood Cliffs, NJ.
- Reznick, D. N., F. H. Rodd, and M. Cardenas. 1996. Life-history evolution in guppies (*Poecilia reticulata*: Poeciliidae). IV. Parallelism in life-history phenotypes. *The American Naturalist* 147:319-338.
- Reznick, D. N., and J. Travis. 1996. The empirical study of adaptation in natural populations. Pp. 243-289 *in* M. R. Rose, and G. V. Lauder, eds. *Adaptation*. Academic Press, San Diego, CA.
- Ricklefs, R. E., and D. B. Miles. 1994. Ecological and evolutionary inferences from morphology: an ecological perspective. Pp. 13-41 *in* P. C. Wainwright, and S. M. Reilly, eds. *Ecological Morphology: Integrative Organismal Biology*. University of Chicago Press, Chicago.
- Rosen, D. E., and R. M. Bailey. 1963. The Poeciliid Fishes (Cyprinodontiformes), their structure, zoogeography, and systematics. *Bull. Am. Mus. Nat. Hist.* N. Y. 126:1-176.

- Shine, R. 2003. Effects of pregnancy on locomotor performance: an experimental study on lizards. *Oecologia* 136:450-456.
- Stanley, S. M. 1979. *Macroevolution: Pattern and Process*. W. H. Freeman and Co., San Francisco.
- Swanson, F. J., S. L. Johnson, S. V. Gregory, and S. A. Acker. 1998. Flood disturbance in a forested mountain landscape. *Bioscience* 48:681-689.
- Van Valen, L. 1974. A natural model for the origin of some higher taxa. *J. Herpetol.* 8:109-121.
- Vitt, L. J. 1981. Lizard reproduction: habitat specificity and constraints on relative clutch mass. *The American Naturalist* 117:506-514.
- Vitt, L. J., and J. D. Congdon. 1978. Body shape, reproductive effort, and relative clutch mass in lizards: resolution of a paradox. *The American Naturalist* 112:595-608.
- Walker, J. A. 1997. Ecological morphology of lacustrine threespine stickleback *Gasterosteus aculeatus* L. (Gasterosteidae) body shape. *Biol. J. Linn. Soc.* 61:3-50.
- Watson, D. M. S., N. W. Timofeeff-Ressovsky, E. J. Salisbury, W. B. Turrill, T. J. Jenkin, R. R. Gates, R. A. Fisher, C. Diver, G. D. H. Carpenter, J. B. S. Haldane, E. W. MacBride, and R. N. Salaman. 1936. A discussion on the present state of the Theory of Natural Selection. *Proceedings of the Royal Society of London. Series B - Biological Sciences* 121:43-73.

Chapter 1

Body shape macroevolution in female *Gambusia* (Poeciliidae): a phylogenetic approach with a comparison of morphometric methods

Abstract: Whether micro-and macroevolutionary processes form a continuum has been contentious. Here we analyze the macroevolutionary pattern of shape variation using a theory that has been largely tested at the microevolutionary level. Biomechanical theory of fish swimming predicts that locomotor cost is reduced with streamlined bodies. However, organisms can deviate from optimal streamlining with respect to reducing costs by having larger caudal regions, a morphology that generates more thrust and should allow for increased burst-swimming performance. Predation and flow are crucial environmental factors for fish, and theory predicts that fish in high-predation sites will have larger caudal areas, and fish in high-flow sites will have more streamlined bodies. Most tests of these predictions have been at the intraspecific level, either with oviparous species or with males of viviparous species (to avoid the complications of pregnancy). Here we perform an interspecific analysis in females of 20 species of *Gambusia*, while taking into account the effect of pregnancy. Several commonly used methods to quantify shape were compared. The effect of pregnancy was analyzed using life history traits gathered through dissections. Phylogenetic relatedness was accounted for by testing our adaptive models using phylogenetically informed statistical methods. As predicted by biomechanical

theory and in agreement with microevolutionary studies, female *Gambusia* showed relatively larger caudal regions in high-predation sites. In contrast to predictions from theory and previous observations, flow had a relatively small influence on shape, only significant for some shape variables and under some models of character evolution. Pregnancy reduced the magnitude of phenotypic divergence between females from contrasting predation and flow regimes. Size and shape, in general, showed strong effect of phylogenetic signal. These results indicate that adaptation to predation, and to a lesser extent flow, is key to body shape evolution in the genus *Gambusia*. Additionally, the importance of phylogenetic history and the role of pregnancy in explaining shape are discussed.

Introduction

The conceptual bridge between micro- and macroevolution has been a contentious topic in evolutionary biology (Arnold et al. 2001). Microevolution refers to evolutionary processes that occur within populations and among conspecific populations, whereas macroevolution refers to larger-scale events among higher taxa (Travis and Reznick 2009). It has been suggested that adaptation at the microevolutionary level can explain macroevolutionary patterns of variation (Darwin 1872: 49; Watson et al. 1936: 58; Charlesworth et al. 1982; Arnold et al. 2001). Under this view, macroevolution is an extension of microevolution (Charlesworth et al. 1982). The support of this view is that a given selective factor would tend to drive phenotypes of different species to similar directions in morphospace (Charlesworth et al. 1982). However, it has been highlighted that alternative evolutionary processes (e.g. random genetic drift, hybridization) can switch the direction of divergence, adding random noise to macroevolutionary patterns (Stanley 1979: 189). This supports the alternative view that microevolutionary divergence cannot be extrapolated to explain all macroevolutionary patterns and processes (Goldschmidt 1940; Van Valen 1974; Eldredge and Cracraft 1980). One strategy to determine the connection between micro- and macroevolution is to use models obtained at the microevolutionary level to predict macroevolutionary patterns (Arnold et al. 2001; Losos and Ricklefs 2009). Here we use that strategy, predicting fish shape at a

macroevolutionary level by using adaptive models largely tested at the microevolutionary level.

According to biomechanical theory, streamlined shapes (forms similar to foils that present little resistance to flow of fluid) minimize drag and thus reduce costs during steady swimming (cruising at constant velocity; McHenry and Lauder 2006). At the other end of the spectrum, bodies with deeper caudal regions generate more thrust by displacing more water during unsteady swimming (motion with linear or angular acceleration; Lighthill 1971; Webb 1982; Walker 1997). (The caudal area is the region where most of thrust is generated in the majority of fishes [Lauder and Tytell, 2006]). A negative correlation between sprint and endurance performance has been observed in fish (Langerhans 2006, 2009b; Oufiero et al. 2011b). Correspondingly, fishes seems to show an inherent morphological trade-off. Fish with larger caudal regions generate more thrust but show reduced streamlining, while fish with tapering caudal areas may increase streamlining but produce less thrust (Langerhans and Reznick 2010). Evidence gathered mostly among populations within species indicates that fish shape aligns along this trade-off in relation to variation among populations in predation and flow, two crucial factors in freshwaters. Fish in high-predation habitats have deeper caudal areas than fish from low-predation sites (Walker 1997; Langerhans and DeWitt 2004; Andersson et al. 2006; Hendry et al. 2006). This is because fish use bursts of unsteady swimming (i.e., rapid production of thrust mostly by the caudal area) to escape from predator strikes (Domenici 2003). Fish

in high-flow sites have more streamlined bodies than those living in low-flow habitats (Langerhans 2008; Langerhans and Reznick 2010), as a way to minimize drag, thereby decreasing the energetic cost of swimming in flow (Aleev 1977). Here we study the effect of both predation and flow on shape at the macroevolutionary level using a comparative approach. We predict that fish from high-predation sites have larger caudal areas, whereas fish from low-predation or high-flow sites have more streamlined bodies. Furthermore, we implement the two most common methods to measure streamlining (Streamlining and Fineness indexes, see Methods) to determine whether or not our results are sensitive to the methods used.

Gambusia is an excellent system to test our ecomorphological hypotheses. The genus currently contains 44 species of small, viviparous, and typically omnivorous fishes, naturally distributed from North America to Northern Colombia, and in the Caribbean west of Puerto Rico (Rosen and Bailey 1963; Lucinda 2003). *Gambusia* have repeatedly colonized a diversity of habitats with distinct predation and flow regimes (Langerhans et al. 2007). Therefore, *Gambusia* can be used as a natural, replicated evolutionary experiment to test the effect of predation and flow at the micro- and macroevolutionary levels. For example, at the interspecific level, Langerhans and Reznick (2010) studied the effect of flow on shape in 12 species of *Gambusia*, observing that males from high-flow sites are more slender and have a lower drag coefficient, characteristics that make them more apt for steady swimming. Here we take a

complimentary approach and study the functional morphology of female *Gambusia* in different flow and predation regimes.

The association between environmental factors and body shape may be more pronounced in females than in males because females bear the extra burden of carrying offspring, and this has been shown to reduce locomotor performance in fishes (James and Johnston 1998; Plaut 2002; Ghalambor et al. 2004), salamanders (Finkler et al. 2003), lizards (Garland 1985; Husak 2006), snakes (Shine 2003; Webb and Lannoo 2004), and mammals (Gittleman and Thompson 1988). This reduction in locomotor performance could be caused by interacting physiological and biomechanical mechanisms. Physiologically, pregnancy can increase the demand for nutrients and oxygen (Plaut 2002), or interfere with physiological functions such as digestion (Weeks 1996). Mechanically, pregnancy can decrease performance during unsteady swimming (an increase of mass reduces the ability to accelerate during burst-starts; Webb 1982) or during steady swimming (an alteration of body shape can increase drag; McHenry and Lauder 2006). It has been suggested that pregnancy can also increase frictional drag by increasing surface area (Ghalambor et al. 2003). We test this idea including surface area as one of the dependent variables analyzed. Previous studies on the functional basis of fish shape have not accounted for the effect of pregnancy of females, or have avoided the issue by analyzing only males. Here we analyze pregnancy by using the difference in shape between females early and late in pregnancy as dependent variables in the analyses.

Accounting for pregnancy is particularly important in this study because the genus *Gambusia* shows remarkable life history variation (e.g. brood size ranges from one to hundreds of embryos per female; Pires et al. 2011). Therefore we predict that pregnancy has an effect on body shape variation associated with swimming performance, by increasing abdominal area and reducing streamlining.

Methods

Source of specimens

Specimens were obtained from museum and personal collections (Table 1, Appendix 1.1). Institutional abbreviation in collection name follows Sabaj-Perez (2010). Among *Gambusia* museum collections we focused on those with a large number of individuals, where habitat could be characterized using field notes (see below), from localities with the least anthropogenic alteration, and obtained during summer (to avoid seasonal effects). A subsample of females was chosen from each collection with the criteria of including the full range of body size and avoiding females with bent or twisted bodies. A total of 46 collections and 20 species were analyzed (Table 1.1). From the total 967 individuals used to calculate minimum size at maturity (see below), 875 fish were retained in further shape analyses after excluding juveniles and bent specimens. The number of collections was reduced to 26 after merging the data of some collections (all from

the respective same species) with similar environmental characteristics. This was done to increase the respective sample size (Table 1.1).

Habitat characterization

We determined predation and flow regimes of each locality (Figure 1.1) by analyzing original field notes, following Reznick et al. (1994). Field notes of each collection were obtained from the online databases of AMNH, NEODAT II, NMNH, and UMMZ. Localities were plotted in Google Earth to obtain geographic coordinates. Flow regime was categorized as low (LF, lakes and ponds) or high (HF, streams). Predation regime of each locality was estimated in three steps. First, the fish community of each site was characterized from field notes (i.e. the list of all other fish collected in that site, Appendix 1.2). Second, the diet of each member of the fish community was obtained from a literature review (Appendix 1.3). Third, localities were classified into two predation regimes, depending on the diet of the highest trophic level of any fish reported for that site. Sites were classified as either low- (LP) or high-predation (HP), depending on the presence of piscivorous fish (Appendix 1.3).

Photographs and Dissections

Prior to dissections, lateral and ventral photographs of specimens were taken following the indications in Zelditch et al. (2004). During dissections we measured standard length of females (SL) using calipers (to the nearest 0.1

mm), fecundity (number of embryos), and stage of development of embryos (see below). Additionally, ovarian tissue dry weight (to the nearest 0.01mg), and mean embryo weight for each female (dry weight of batch of embryos/fecundity, to the nearest 0.01 mg) were measured for a parallel study on the life history of the genus (Torres-Mejia, unpub.). Stage of development of embryos was quantified using the scale of Haynes (1995), as modified by Pires et al. (2007); these stages range from egg (stage 0) to fully-formed embryo ready for birth (stage 50). Minimum size at maturity was estimated for each collection as the SL of the smallest female with stage of development of embryos ≥ 2 .

Geometric Morphometrics

Shape variation was analyzed with two-dimensional, landmark-based geometric morphometrics (GMM; Zelditch et al. 2004). GMM is a quantitative method to analyze shape variation using coordinates of points located in distinct positions along the body. Coordinates of multiple specimens are superimposed to extract shape variation independent of location, scale, and rotation of the specimens in the images. TpsDig2 2.12 (Rohlf 2008) was used on lateral pictures to digitize 10 landmarks and 3 semilandmarks (Figure 1.2 B). Semilandmarks (landmarks that define non-homologous positions along the outline) were slid to minimize the bending energy criterion, using methods outlined in Zelditch et al (2004), and R-code adapted from Morpho (Stefan Schlager, unpub). Procrustes superimposition was performed using R-code adapted from Claude (2008).

The effect of intraspecific variation in female size and pregnancy condition (within each sample) was accounted for by estimating shape configuration at two stages: early in pregnancy (when embryos were at developmental stage 0) and late in pregnancy (embryos at stage 50). In both cases, body size was equal to the average centroid size (the square root of the sum of distances between each landmark and their centroid; Zelditch et al. 2004). These two extreme morphologies were estimated with a multiple regression model (Monteiro 1999; Berner 2011) for each collection, having projected landmark positions as dependent variables, and centroid size and stage of development of embryos as the predictors. The resulting size- and pregnancy-standardized geometric morphometric configurations were used for further analyses. The effect of pregnancy condition on shape and the interaction of that effect with size, predation, and flow was quantified by subtracting superimposed configurations early in pregnancy from those late in pregnancy. The resulting differential configurations were used in further analyses. These differences were visualized by adding them onto the average female configuration.

Each of the three geometric morphometric configuration sets (early in pregnancy, late in pregnancy, and their difference) was analyzed with a Principal Components Analyses (PCA) to reduce dimensionality. Principal Components (PC) that accumulated more than 99% percent of variation were retained in further analyses. Differences in shape were visualized using vectors with length,

thickness and darkness that indicate the magnitude of variation between superimposed shapes with R-code derived from Claude (2008).

Streamlining and Areas

We measured streamlining using the streamlining Index (SI, McHenry and Lauder 2006), and the fineness index (FI; Langerhans and Reznick 2010). SI indicates the similarity in the distribution of mass of a fish in comparison with the optimal distribution of mass of a streamlined foil of the same maximal length, width, and depth (McHenry and Lauder 2006). A SI value of one indicates an optimal distribution of body mass along the longitudinal axis of the fish.

Deviations from optimal mass distribution may come from having more mass towards the head ($SI < 1$) or more mass towards the tail ($SI > 1$). FI quantifies the body elongation of a fish, ranging from the asymptotic value of 0 (a infinitely elongated body) to one (an optimally elongated body, its length 4.5 times its depth). A custom code was implemented to calculate SI and FI using Matlab 7.5 with Image Processing Toolbox 6.0 (MathWorks, Natick, MA). The 3D surface area of each fish (SA) was calculated digitally in the same Matlab code. The relative size of the caudal peduncle was calculated as the 2D caudal peduncle area (CP, Figure 1.2B) over 2D lateral body area. These areas were measured by taking advantage of the landmarks digitized for GMM analysis. Each area was estimated as the sum of the areas of triangles whose vertices were the

respective enclosing landmarks. See further details about estimation of streamlining variables in Appendix 1.4.

The effect of intraspecific variation in female size and pregnancy condition was accounted for using multiple regression models, as was done in the GMM analysis. In this case SI, FI, SA, and CP were the dependent variables, and female size was quantified as log-transformed SL. The resulting size- and pregnancy-standardized variables were used for further analyses. The effect of pregnancy on these morphological variables was tested by using the difference between the values early and late in pregnancy, as was done in the GMM analysis. Therefore, in the adaptive models explained below, we analyzed three values for each morphological variable: early in pregnancy, late in pregnancy, and their difference.

Statistical analysis: testing the independence of predation and flow

The independence of predation and flow was evaluated prior to shape analyses, using phylogenetic independent contrasts (PIC; Felsenstein 1985; Garland et al. 1992). Accounting for phylogenetic relatedness when analyzing correlation of environmental regimes is justified because closely related species tend to live in similar ecological environments (Losos 2008), an argument made before (Garland et al. 1992; Oufiero et al. 2011a).

Statistical analysis: adaptive models

We first analyzed size as a dependent variable of predation and flow, using the models:

$$size = predation + \varepsilon$$

$$size = flow + \varepsilon$$

$$size = predation + flow + \varepsilon$$

$$size = predation + flow + predation*flow + \varepsilon$$

where *predation* and *flow* are the predictor variables, ε is the error term, and *size* was measured in two ways, as the mean centroid size and as log-SL. Similarly, for each morphological variable we tested five adaptive hypotheses:

$$morphology = size + \varepsilon$$

$$morphology = size + predation + \varepsilon$$

$$morphology = size + flow + \varepsilon$$

$$morphology = size + predation + flow + \varepsilon$$

$$morphology = size + predation + flow + predation*flow + \varepsilon$$

where *morphology* represents each morphological variable (SI, FI, SA, CP, and each of the three PC scores), *size* was a covariate (centroid size when the dependent variables were GMM variables, and log-SL in other cases), *predation* and *flow* are the predictor variables, and ε is the error term.

Adaptive models were evaluated using a phylogenetic framework to account for the potentially hierarchical structure of the data. We fulfilled this with two different methods, depending on whether the dependent variables were

GMM variables or not. In the first case, we used phylogenetic MANCOVAs (Garland et al. 1993; Revell et al. 2007) because they can handle multiple dependent variables (in this case PC scores), while accounting for covariates (body size), when factors are tested (in this case predation and flow). Two models of character evolution were tested, Ordinary Least Squares (OLS, equivalent to a standard, non-phylogenetic MANOVA that assumes all samples are equally related in a star-phylogeny; Garland et al. 2005), and Brownian Motion (BM, random divergence). For the BM model, the probability of the F-statistics obtained from OLS was calculated using empirical null distributions. These distributions were calculated from Monte Carlo simulations of tip data (1,000 simulations), using the original topology as the phylogenetic structure, and observed root values as starting values. P-values were the proportion of times that the observed F-statistic was larger than the simulated F-values.

Shape variation explained by MANCOVA predictors was visualized using a canonical analysis as explained in Langerhans (2009b). This method of visualization of group differences avoids the distortion of shape space that would occur if canonical values from MANCOVA were directly analyzed (Langerhans 2009b; Mitteroecker and Bookstein 2011). First, we extracted the Sum of Squares and Cross-Products matrix (SSCP) for each predictor (size, predation, flow, and predation*flow) from MANCOVA. Second, an eigenanalysis of the SSPC produces $n-1$ eigenvectors, where n is the number of levels of the respective factor. Third, eigenvector values were multiplied by the matrix of PC

scores to produce a divergence vector, which is a linear combination of the PC scores that has the highest correlation with the respective factor in Euclidean space. Fourth, shapes associated with the mean divergence vector values for each factor level were calculated by regressing divergence vector values against PC scores. The phylogenetic signal (K ; Blomberg et al. 2003) of each canonical vector, and the probability that each K was larger than the null hypothesis of no signal ($K=0$) were calculated using *phylosignal* in the R package *picante* (Kembel et al. 2010). This probability is calculated as the frequency that the observed phylogenetically correct mean (MSE) was larger than n randomized MSE. In this study $n=9,999$.

When dependent variables were non-GMM variables, we accounted for phylogeny using phylogenetic Generalized Least Squares (GLS; Grafen 1989). This method is a linear model that structures the error term as a multiple of the variance-covariance matrix of phylogenetic relatedness (Martins and Hansen 1997; Garland and Ives 2000; Rezende and Diniz-Filho 2012). Before running GLS, we plotted PIC of each trait to detect the presence of potentially influential points, which were never observed. Three models of character evolution (i.e. structure of the error) were evaluated for each GLS adaptive model. The first model of character evolution was OLS (non-phylogenetic, assuming phylogenetic signal in the residuals is null). The second model (RegPagel) is a regression model that assumes Brownian motion (Pagel 1999; Garland et al. 2005), and simultaneously estimates the parameter coefficients and λ , a measurement of

phylogenetic signal (i. e. the effect of shared evolutionary history in phenotypic variation; Blomberg et al. 2003). Algebraically, λ is a factor that multiplies the off-diagonal elements of the variance-covariance matrix of relatedness (Freckleton et al. 2002). The value λ ranges from zero, indicating phylogenetic independence equivalent to a star phylogeny, to one, indicating covariation of traits fully proportional to shared phylogenetic history. The third model was the Ornstein–Uhlenbeck process (RegOU), which assumes Brownian motion that tends to drift towards an optimum determined by selection (Hansen 1997; Butler and King 2004). As in RegPagel, RegOU also allows the simultaneous estimation of regression coefficients and a measure of phylogenetic signal, in this case the parameter d (Lavin et al. 2008). The value of d also indicates the optimal stretching of the branch lengths. When d is less than one the tree is less hierarchical and more like a star-phylogeny (i.e. nodes pulled towards the root). When d is larger than one the tree is more hierarchical than the original (i.e. nodes pulled towards the tips).

To select among the multiple models for each dependent variable we used model selection with model averaging (Burnham and Anderson 2002). First, small sample corrected Akaike information criterion (AIC_c; Anderson and Burnham 2002; Hansen et al. 2008) was calculated for each set of models for each GLS. AIC_c differences (Δ_i), the difference in AIC_c values between each model and the best supported one (the one with the lowest AIC_c), were used to estimate the Akaike weights (w):

$$w_i = \frac{\exp(-0.5\Delta_i)}{\sum_{j=1}^J \exp(-0.5\Delta_j)}$$

where J is the total number of models. Akaike weights are Bayesian posterior probabilities (i.e. model probabilities) that indicate the amount of evidence explained by each model (Anderson 2008), ranging from zero (model not explaining variation) to one (model fully explaining variation). Evidence Ratios, the number of times the best model is were estimated Evidence Ratio (ER) equals the w the most-supported model of the set over each model's w . It indicates the number of times the best model has more evidence than each model (Anderson 2008). Average coefficients and their standard errors were calculated as the values obtained from each model, weighted with their respective w_i . Model averaging was performed with unconditional estimation (using all models instead of only those including each parameter; Anderson 2008), which is a better alternative when the objective is to determine the strength of the response variable predictors (Grueber et al. 2011). There is some debate about the use of unconditional estimation as opposed to conditional estimation (considering only the models that included a parameter to calculate its average coefficient; Grueber et al. 2011). We performed estimation with both methods and found few substantial differences; therefore we show here only unconditional estimations. We did not attempt to include more models in our

model sets because of our small sample size. A rule of thumb in model selection procedures is that the number of models should not exceed the sample size (Anderson 2008). In this study, the sample size was 26, and for each dependent variable we tested 25 models.

Statistical analysis: phylogeny of *Gambusia*

The *Gambusia* phylogeny used in these analysis (B. Langerhans, unpub.) is available in Appendix 1.5. The phylogeny was constructed using majority (50%) consensus, and is based upon two mitochondrial (ND2, Cyt b) and one nuclear (S7) gene. Branches were transformed using two methods. In the first method the phylogeny had branch lengths proportional to the units of inferred nucleotide substitution (Appendix 1.6). When we had data for multiple populations per species, we added new branches with length 0.00461, which was chosen because this was the average distance between pairs of conspecific *Gambusia* populations (B. Langerhans, unpub.). Modeling error covariance with a tree that has branch lengths proportional to nucleotide substitution assumes that the variance of evolutionary change is proportional to the amount of divergence in the genes used to reconstruct the phylogeny (Oufiero et al. 2011a). This method contrasts with the common recommendation of using a tree where tips are contemporaneous for extant species or populations (i.e. ultrametric), which assumes that the expected variance of evolutionary change is proportional to time. We repeated all analyses using the ultrametric tree obtained by stretching

the branches with a penalized likelihood algorithm, using function *chronopl* (with *lambda* set to one) in the R package *ape* (Paradis et al. 2004). The results were qualitatively the same as the results with the original branch lengths; therefore, here we only show the latter results.

In the second phylogenetic transformation, branch lengths were set to a constant value of one (Appendix 1.7). This reduces magnification of phenotypic differences between populations of the same species. Magnification occur because phylogenetic distance is used to weight phenotypic differences; such magnification could be exaggerated when comparing closely related taxa, the case of populations of the same species. However, this branch length transformation implicitly assumes that phenotypic divergence occurs at speciation events and/or as populations split from each other.

Soft-polytomies (multifurcations resulting from lack of resolution at a given node) in the available phylogeny were resolved prior to analysis by making the tree dichotomous using the function *multi2di* in the package *ape* (Paradis et al. 2004). These newly resolved branches were manually set to zero length. Type I error produced by soft polytomies was corrected by reducing the degrees of freedom of the denominator when calculating the significance of F-values for each coefficient of phylogenetic regression models. The reduced degrees of freedom were equal to $N-k-1-z$, where N are the number of tips in the phylogeny, k the number of parameters estimated for the respective model, and z are the

number of branches that were set to zero length (Garland and Díaz-Uriarte 1999), in this study $z = 5$.

Except for GLS regressions, which were performed using the Matlab program RegressionV2.m (A. R. Ives and T. Garland Jr.; Lavin et al. 2008), all analyses were executed using code written in R (R Development Core Team 2011).

Results

Predation and flow showed low but significant phylogenetic signal (predation: $K=0.179$, $p=0.036$; flow: $K=0.195$, $p=0.008$). PIC of predation and flow were negatively correlated ($r=-0.75$, $p<0.001$).

GMM variables

PCs from GMM indicated a considerable range of body-shape variation among *Gambusia* females (Appendix 1.8-1.10). As expected, the main effect of pregnancy on shape was a distension of the abdominal area (Appendix 1.8). Size partially explained shape of females early in pregnancy; females of larger species have deeper bodies posterior to the opercle and shorter caudal peduncles (Figure 1.3, first row). This effect disappears late in pregnancy because there is a tendency for smaller females to have a more extended abdomen when pregnant (Table 1.3). The pregnancy-size interaction effect was

correlated with SI, suggesting that larger species have relatively less abdominal mass late in pregnancy.

The effect of predation on shape was correlated with streamlining: high-predation species showed a displacement of lateral area from the dorsal profile towards the caudal area (Table 1.3; Figure 1.3, second row). This effect was correlated with SI, corroborating that there is a displacement of mass towards the caudal area. The effect of predation was statistically significant under the three phylogenetic scenarios. There was a tendency for an interaction between predation and pregnancy: high-predation species tend to have more distended abdomens late in pregnancy.

Flow regime explained very little of the variation in shape, with only a tendency for high-flow species to be more elongated (Table 1.3; Figure 1.3, third row). This tendency was significant only in females early in pregnancy when accounting for phylogeny (under the BM model with original branch lengths, $p=0.016$). There was an interaction between flow and pregnancy, with high-flow species having slightly more distended abdomens. This interaction was significant only when accounting for phylogeny (under BM with original branch lengths, $p=0.018$). There was also an interaction between predation and flow, with LP species being more elongated in LF sites, and vice versa in HF sites (Table 1.3; Figure 1.3, fourth row). This interaction was not influenced by pregnancy, but it was significant only when accounting for phylogeny (under BM model with original branch lengths).

Non-GMM variables

None of the GLS models for each dependent variable had a distinctly high AIC_c value (Appendix 1.5), or accounted for a high proportion of evidence (Table 1.3, w values). However, average models are qualitatively similar models to the best-supported model for each dependent variable (Table 1.3). The best-supported phylogenetic models were most often the ones with branch lengths proportional to molecular distance, rather than constant branch lengths (Table 1.3).

Variation in body size showed high levels of phylogenetic signal and was not related with predation or flow, neither when size was measured as log SL or as CS (Table 1.3). SI early in pregnancy was independent of body size, but related to predation (Tables 1.3 and Appendix 1.11). This effect of predation disappeared late in pregnancy because HP species showed a greater reduction in streamling with pregnancy, indicating that they had a larger abdominal distension when pregnant (Figure 1.4). LP species had SI values lower than the optimal, whereas the range of SI values in HP species included optimal values during intermediate stages of pregnancy (Figure 1.4). Most SI models showed high levels of phylogenetic signal, including the interaction between pregnancy and predation effect (Table 1.3 and Appendix 1.11).

FI showed a tendency to be negatively related with body size, implying that larger females diverge from the optimal fineness by having deeper bodies. There was a clear interaction between size and pregnancy, with larger species being more optimally elongated when pregnant as compared with smaller

species. FI was not related to predation, flow, or their interaction. In general, models that have FI as the dependent variable showed relatively low levels of phylogenetic signal (Table 1.3 and Appendix 1.11).

Both SA and CP were strongly related to body size but not to predation or flow (Table 3). Not surprisingly, SA was related to pregnancy (reflecting the abdominal distension late in pregnancy), and CP was not (caudal peduncle area does not change with pregnancy). Regressions with SA and CP showed moderate levels of phylogenetic signal in the residuals (Table 3 and Appendix 1.11).

Discussion

This study is one of only a handful that have tested the ecomorphological predictions of biomechanical theory in female fishes while accounting for pregnancy, and the first to do so at the interspecific level using phylogenetically informed statistical methods. Furthermore, this study is unique in simultaneously analyzing the effects of predation and flow, two main selective factors in freshwaters. We found that predation, and to a lesser extent flow, were related to body shape of *Gambusia* females in a manner predicted by biomechanical theory. Some of the shape variation was explained by an interaction between predation and flow. Pregnancy interacted with predation, reducing the divergence among predation regimes when females are late pregnancy.

Our results at the macroevolutionary level largely match the previous observations at the microevolutionary level, suggesting a continuum from micro- to macroevolution. However the proportion of shape variation explained by our adaptive hypothesis was relatively small compared to the large range of morphological variation observed. This may be indicative that divergence is not necessarily larger at the macroevolutionary level, as suggested before (Bell 1995). This phenotypic stabilization could represent a peak in the adaptive landscape, reached by either micro- or macroevolutionary divergence.

This study's conclusions have two main limitations. First, we analyzed field-caught specimens, which may be showing variation that is correlated with unmeasured environmental factors. This could be seen as a positive quality, that despite the possible noise coming from unmeasured factors we still found a significant effect of predation, and in less extend flow. But it also brings the question of whether these differences would remain if the fish had been maintained in a common garden (i.e., whether the phenotypic differences have a plastic or a genetic basis; Garland and Adolph 1991). Previous studies on *Gambusia* lead us to consider that the body shape differences observed have a considerable genetic basis. In *G. affinis* (Langerhans et al. 2004) and *G. hubbsi* (Langerhans 2009a), differences in shape among wild populations of each species were still observed in common garden experiments. However, a common garden study would clarify this question for body shape of female *Gambusia*.

A second limitation of this study is that we are assuming that the body shape observed is a good proxy of locomotor ability. Moreover, we assumed that the locomotor traits presumably correlated to shape are related with performance. Measuring performance is important because it is the direct subject for the action of selection (Arnold 1983). However, there is evidence that our observations on body shape is the kind that have repercussions on performance and fitness. Previous studies in fish evidence the correlation between caudal region area and thrust (Langerhans et al. 2004; Langerhans 2009a), and between optimal streamlining and higher endurance (Langerhans and Reznick 2010), and lower inertial drag coefficients (McHenry and Lauder 2006). Moreover, acceleration ability causes high survivorship during predatory strikes in *G. hubbi* (Langerhans 2009a). Anyhow, a study of shape, performance (e.g., Langerhans 2009a), and fitness in female *Gambusia* would shed light on this issue.

Body size effect on shape

Females of *Gambusia* species show a relatively narrow range of body sizes (mean collection body size: 22.48-43.17 mm SL; Chapter 2). Nevertheless, body size was a good predictor of most body shape variables. The main allometric effect on shape was that larger species had deeper bodies posterior to the opercle, and shorter caudal peduncles. This effect was not correlated with SI, indicating that body size in *Gambusia* varies without affecting streamlining (a

proxy of minimization of drag). Furthermore, larger bodies showed less shape change with pregnancy, possibly because larger females have more abdominal space available for a given gonadal mass. If the change in shape produced by pregnancy increases drag when swimming, then having larger bodies may minimize this particular aspect of the cost of pregnancy in this genus.

Correlation of predation and shape

Here we provide interspecific evidence that fish shape responds evolutionarily to predation as predicted by biomechanical theory - that is, fish exposed to HP environments have relatively larger caudal regions than their LP counterparts. However, *Gambusia* females attained this morphology in an unexpected manner. Whereas most fish living in HP sites have smaller heads and larger caudal peduncles (Walker 1997; Langerhans and DeWitt 2004; Langerhans et al. 2004; Hendry et al. 2006), HP *Gambusia* did not have larger caudal peduncles and instead showed a displacement of dorsal mass toward the postero-ventral direction. The direct measurement of caudal peduncle area from lateral pictures did not match the SI results, indicating that estimating relative caudal peduncle area is not necessarily a good proxy for estimating caudal peduncle mass, a variable directly linked to swimming power output (Altringham and Ellerby 1999). Theoretically, fish with deeper caudal peduncles displace more water when undulating, and therefore generate more thrust (Lighthill 1971; Lauder and Tytell 2006). At the same time, a larger mass requires larger power thrust to produce

displacement (Lauder and Tytell 2006). Therefore, a net gain in power thrust could be obtained by an increase in caudal peduncle depth or by a reduction in the anterior mass, the latter being the case of *Gambusia* females. Future studies will be required to determine if these populations and species actually differ in swimming performance, as would be expected from the differences in body shape.

Unexpectedly, LP females were not more streamlined. Early in pregnancy, both LP and HP females deviated from the optimal SI values, the former because of their bulkier abdomens, the latter because of their relatively large caudal region. Pregnancy displaced LP females even more from optimality, whereas HP females passed through optimal streamlining at intermediate stages of pregnancy. This may indicate that shape of LP species could be responding to other selective pressures that require larger abdomens. Perhaps LP species may have larger digestive systems because of a more herbivorous diet as occurs in guppies (Zandonà et al. 2011). In pregnant HP females, it is unclear whether their more streamlined bodies are an incidental by-product of pregnancy or an adaptation to minimize locomotion costs in their demanding environments. It would be worthwhile testing whether the enhanced streamlining of pregnant HP females allows for better steady-swimming performance since the only study that has compared the effect of pregnancy on fish swimming performance focused on unsteady swimming (Ghalambor et al. 2004).

Weak correlation between flow and body shape

A recent review on the association between flow regime and body shape (Langerhans 2008) found that in about 75% of studies fish from HF habitats are more streamlined. That result was consistent across phylogeny and at both intra- and interspecific levels. We found the same trend in our GMM analysis of *Gambusia* females, but the effect was rather weak (HF females were more elongated, but the effect was not significant). We can rule out our lack of a highly significant result being due to a lack of phenotypic variation in body depth because such variation was observed (Appendix 1.8-1.10). Instead, this difference in magnitude could be an outcome of two factors. Firstly, predation and flow were negatively correlated, and correlation of predictor variables in a linear model reduces their estimated effect size (Tabachnick and Fidell 2001). It is possible that the effect of predation and flow were partially overlapping, given that the biomechanical predictions were opposite for the two regimes (i.e. HF and LP females were expected to be more streamlined, and HF frequently had LP regimes). However, our GLS results indicate that this correlation did not obscure the effect of flow because model selection showed that the models where size and flow were the only predictors had considerably less support (Appendix 1.11).

Secondly, the classification of flow regimes was a rather rough description of flow. Most studies, including this one, contrast lotic (streams) vs. lentic (lacustrine) habitats. The problem with this straightforward contrast is that rivers and lakes also differ, in addition to water velocity, in many other aspects, such as

benthic biota and structure, physical and chemical parameters, and productivity (Wetzel and Likens 2000). Moreover, neither lentic nor lotic habitats are homogeneous. Lentic habitats may have complex benthic areas where the key factor is maneuverability (Domenici 2003), which is higher in fish with deeper bodies (Webb 1984). Lotic habitats vary spatially in flow regime, from mountain streams structured as series of step-pool and pool-riffle patterns (Wohl and Merritt 2008) to lowland rivers with sinuous streambeds and lateral floodplain expansions (Church 2002). Lotic habitats also vary temporally, from streams whose flow rarely changes to the more typical streams that periodically flood (Sabo and Post 2008). These changes in flow can be characterized by their frequency, magnitude, seasonality, and timing, among other factors (Poff et al. 1997; Sabo and Post 2008). It would have been ideal for our study to have characterized flow in this way; however, those methods are very data-demanding (e.g., the recommended length of daily flow time series is 20 years; Sabo and Post 2008), and such flow data were not available.

We show that female *Gambusia* have a different evolutionary shape response to flow-regime than male *Gambusia* (Langerhans and Reznick 2010). Whereas we observed that flow explained a modest amount of shape variation, Langerhans and Reznick (2010) observed that male *Gambusia* living in HF regimes were clearly more elongated. The differences between these two studies could lie in intersexual differences in divergence patterns or in methodological differences between the studies. Regarding the first option, sexes may (Gilchrist

et al. 2004; Berner et al. 2008; Blob et al. 2008) or may not (Butler et al. 2000; McGuigan et al. 2003; Hendry et al. 2006) respond in parallel to a given type of selection (Garland et al. 2011). A joint analysis using both sexes while accounting for pregnancy could be fruitful. Regarding methodology, there are three main differences between our study and that of Langerhans and Reznick (2010). First, there was only partial overlap in the species sampled. Second, the phylogeny used here was a more updated version of the one used in the previous study, although this effect is expected to be small because comparative methods are relatively robust to minor changes in phylogenetic topologies (Díaz-Uriarte and Garland 1998). And third, predation regime was not included as a factor in the previous study. Analyzing predation and flow simultaneously proved particularly important in this study because these factors were correlated, as was discussed above.

Importance of pregnancy

We found that pregnancy generally had the effect of increasing abdominal size in *Gambusia* females. This is not surprising because species of *Gambusia* produce large batches of embryos that increase in wet mass through development (Chapter 2). More interestingly, pregnancy had the effect of reducing the presumably adaptive differences in shape across the gradient of predation. Early in pregnancy, HP females clearly had relatively larger caudal peduncles. Pregnancy increased the mass in the anterior portion of the body (the abdomen).

However, the increase was estimated to be larger for HP females, reducing the differences in shape between LP and HP. Two previous studies at the intraspecific level found that pregnancy reduces adaptive divergence, the same result we found at the interspecific level. Early in pregnancy, guppies from HP localities have faster burst-starts than LP guppies, a pattern reversed late in pregnancy (Ghalambor et al. 2004). *Brachyrhaphis rhabdophora* (Poeciliidae) from HP sites have larger caudal peduncles and more elongated bodies than those from LP sites, but only early in pregnancy, as late in pregnancy those differences disappeared (Wesner et al. 2011). Therefore, there seems to be a widespread phenomenon of pregnancy reducing adaptive divergence in body shape in response to predation.

Phylogenetic effect on evolution of shape in female *Gambusia*

The two main environmental variables analyzed here, predation and flow, showed phylogenetic signal and were negatively correlated. This indicates niche conservatism in this genus (i.e., related species tend to live in similar habitats). Similarly, a fair amount of size and shape variation was explained by phylogenetic relatedness (d and λ in Table 1.3 and Appendix 1.11), another example of the ubiquity of phylogenetic signal and its high levels in morphological characters (Blomberg et al. 2003). Significant levels of phylogenetic signal were found when analyzing both GMM and functional variables for shapes early and late in maturity. In contrast, the difference between shapes early and late in

pregnancy generally showed low phylogenetic signal, implying that the way female shape changes throughout pregnancy is independent of phylogeny. The exception was the effect of pregnancy on SI, which was better explained by RegPagel models with high phylogenetic signal. Therefore, the manner in which females vary in the distribution of their mass with pregnancy in response to predation has a strong phylogenetic component in *Gambusia*.

Sensitivity of results to methods used to measure shape

Here we measured SI, FI, relative caudal peduncle area, and PCs from geometric morphometrics. Most papers on ecomorphology of fish shape typically implement one of these methods. By including all of these measures in the same study we demonstrate that these methods are not interchangeable. Streamlining is commonly measured with FI (or a similar body-to-depth proportion index), perhaps because of its simplicity. FI has some support in biomechanical theory in that the most-streamlined shapes are 4.5 times deep as long (Blake 1983). However, that optimum is not a critical value (i.e., does not have a narrow peak), as fish with a wide range of body proportions (from two to six times deep as long) have very similar drag coefficients (Blake 1983). Moreover, a fish could have an optimal body-to-depth ratio but still have a shape far from streamlined because FI does not take into account the location of the maximal depth. An object as blunt as a mallet could still show the optimal fineness proportion of 4.5. McHenry and Lauder's (2006) SI is a better alternative to measure streamlining because it is

based on the comparison of a body with empirically tested foils. Additionally, the expected correlation between SI and swimming performance has been supported empirically, as optimal SI values were related to higher endurance in *Gambusia affinis* (Langerhans and Reznick 2010), and with lower inertial drag coefficients in *Danio rerio* (McHenry and Lauder 2006).

Geometric morphometrics is very good at estimating axes of morphological variation (Zelditch et al. 2004) but may not necessarily extract variation that is most closely related to biomechanical function. Configuration in shape space is typically used to calculate axes of correlated variation (equivalent to Principal Components). The resulting vectors of variation may or may not be related to streamlining. Most studies on fish ecomorphology typically inspect thin-plate splines of PCs looking for shapes similar to the morphology of interest. However, such qualitative inspections are poor estimations of how close the fish matches a shape that optimizes a functional trait. Regarding streamlining in particular, a body may look streamlined in one perspective (e.g. lateral view), but reveal mass distributions that depart from streamlining in other perspectives. In this study, we were able to visualize difference in shape using GMM, and at the same time we found their functional significance by correlating GMM variables with SI (Table 1.2). We suggest that geometric morphometrics can be complementary to direct calculation of streamlining, and that both methods should be performed in conjunction when studying body shape in fishes.

Literature Cited

- Aleev, I. G. 1977. Nekton. Dr. W. Junk b. v. Publishers, The Hague.
- Altringham, J. D., and D. J. Ellerby. 1999. Fish swimming: patterns in muscle function. *J. Exp. Biol.* 202:3397-3403.
- Anderson, D. R. 2008. Model Based Inference in the Life Sciences: A Primer on Evidence. Springer, New York, NY.
- Anderson, D. R., and K. P. Burnham. 2002. Avoiding pitfalls when using information-theoretic methods. *The Journal of Wildlife Management* 66:912-918.
- Andersson, J., F. Johansson, and T. Söderlund. 2006. Interactions between predator- and diet-induced phenotypic changes in body shape of crucian carp. *Proceedings of the Royal Society B: Biological Sciences* 273:431-437.
- Arnold, S. J. 1983. Morphology, performance and fitness. *Am. Zool.* 23:347-361.
- Arnold, S. J., M. E. Pfrender, and A. G. Jones. 2001. The adaptive landscape as a conceptual bridge between micro- and macroevolution. *Genetica* 112-113:9-32.
- Bell, M. A. 1995. Intraspecific systematics of *Gasterosteus aculeatus* populations: implications for Behavioral Ecology. *Behaviour* 132:1131-1152.
- Berner, D. 2011. Size correction in biology: how reliable are approaches based on (common) principal component analysis? *Oecologia* 166:961-971.

- Berner, D., D. C. Adams, A. C. Grandchamp, and A. P. Hendry. 2008. Natural selection drives patterns of lake–stream divergence in stickleback foraging morphology. *J. Evol. Biol.* 21:1653-1665.
- Blake, R. W. 1983. *Fish locomotion*. Cambridge University Press, Cambridge.
- Blob, R. W., W. C. Bridges, M. B. Ptacek, T. Maie, R. A. Cediell, M. M. Bertolas, M. L. Julius, and H. L. Schoenfuss. 2008. Morphological selection in an extreme flow environment: body shape and waterfall-climbing success in the Hawaiian stream fish *Sicyopterus stimpsoni*. *Integr. Comp. Biol.* 48:734-749.
- Blomberg, S. P., T. Garland, Jr, and A. R. Ives. 2003. Testing for phylogenetic signal in comparative data: behavioral traits are more labile. *Evolution* 57:717-745.
- Burnham, K. P., and D. R. Anderson. 2002. *Model selection and multimodel inference: a practical information-theoretic approach*. Springer Verlag, New York, NY.
- Butler, M. A., and A. A. King. 2004. Phylogenetic comparative analysis: a modeling approach for adaptive evolution. *Am. Nat.* 164:683-695.
- Butler, M. A., T. W. Schoener, and J. B. Losos. 2000. The relationship between sexual size dimorphism and habitat use in Greater Antillean *Anolis* lizards. *Evolution* 54:259-272.
- Charlesworth, B., R. Lande, and M. Slatkin. 1982. A Neo-Darwinian commentary on macroevolution. *Evolution* 36:474-498.

- Church, M. 2002. Geomorphic thresholds in riverine landscapes. *Freshwat. Biol.* 47:541-557.
- Claude, J. 2008. *Morphometrics with R*. Springer, New York.
- Darwin, C. 1872. *On the origin of species by means of natural selection*. Murray, London, UK.
- Díaz-Uriarte, R., and T. Garland, Jr. 1998. Effects of Branch Length Errors on the Performance of Phylogenetically Independent Contrasts. *Syst. Biol.* 47:654-672.
- Domenici, P. 2003. Habitat, body design and the swimming performance of fish. Pp. 137–160 *in* V. L. Bels, J. P. Gasc, and A. Casinos, eds. *Vertebrate Biomechanics and Evolution*. BIOS Scientific Publishers Ltd, Oxford.
- Eldredge, N., and J. Cracraft. 1980. *Phylogenetic Patterns and the Evolutionary Process*. Columbia University Press, New York, NY.
- Felsenstein, J. 1985. Phylogenies and the Comparative Method. *The American Naturalist* 125:1-15.
- Finkler, M. S., M. T. Sugalski, D. L. Claussen, and S. J. Beaupre. 2003. Sex-related differences in metabolic rate and locomotor performance in breeding spotted salamanders (*Ambystoma maculatum*). *Copeia* 2003:887-893.
- Freckleton, R. P., P. H. Harvey, and M. Pagel. 2002. Phylogenetic analysis and comparative data: a test and review of evidence. *Am. Nat.* 160:712-726.
- Garland, T., A. F. Bennett, and E. L. Rezende. 2005. Phylogenetic approaches in comparative physiology. *J. Exp. Biol.* 208:3015-3035.

- Garland, T., Jr. 1985. Ontogenetic and individual variation in size, shape and speed in the Australian agamid lizard *Amphibolurus nuchalis*. *J. Zool.* 207:425-439.
- Garland, T., Jr, and S. C. Adolph. 1991. Physiological differentiation of vertebrate populations. *Annu. Rev. Ecol. Syst.* 22:193-228.
- Garland, T., Jr, and R. Díaz-Uriarte. 1999. Polytomies and phylogenetically Independent Contrasts: examination of the bounded degrees of freedom approach. *Syst. Biol.* 48:547-558.
- Garland, T., Jr, A. W. Dickerman, C. M. Janis, and J. A. Jones. 1993. Phylogenetic analysis of covariance by computer simulation. *Syst. Biol.* 42:265-292.
- Garland, T., Jr, P. H. Harvey, and A. R. Ives. 1992. Procedures for the analysis of comparative data using phylogenetically independent contrasts. *Syst. Biol.* 41:18-32.
- Garland, T., Jr, S. A. Kelly, J. L. Malisch, E. M. Kolb, R. M. Hannon, B. K. Keeney, S. L. Van Cleave, and K. M. Middleton. 2011. How to run far: multiple solutions and sex-specific responses to selective breeding for high voluntary activity levels. *Proceedings of the Royal Society B: Biological Sciences* 278:574-581.
- Garland, T., Jr., and A. R. Ives. 2000. Using the past to predict the present: confidence intervals for regression equations in phylogenetic comparative methods. *The American Naturalist* 155:346-364.

- 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*). *The American Naturalist* 164:38-50.
- Ghalambor, C. K., J. A. Walker, and D. N. Reznick. 2003. Multi-trait selection, adaptation, and constraints on the evolution of burst swimming performance. *Integr. Comp. Biol.* 43:431-438.
- Gilchrist, G. W., R. B. Huey, J. Balanya, M. Pascual, and L. Serra. 2004. A time series of evolution in action: a latitudinal cline in wing size in South American *Drosophila subobscura*. *Evolution* 58:768-780.
- Gittleman, J. L., and S. D. Thompson. 1988. Energy Allocation in Mammalian Reproduction. *Am. Zool.* 28:863-875.
- Goldschmidt, R. 1940. *The material basis of evolution*. Yale University Press, New Haven, CT.
- Grafen, A. 1989. The Phylogenetic Regression. *Philosophical Transactions of the Royal Society of London. Series B, Biological Sciences* 326:119-157.
- Grueber, C. E., S. Nakagawa, R. J. Laws, and I. G. Jamieson. 2011. Multimodel inference in ecology and evolution: challenges and solutions. *J. Evol. Biol.* 24:699-711.
- Hansen, T. F. 1997. Stabilizing selection and the comparative analysis of adaptation. *Evolution* 51:1341-1351.

- Hansen, T. F., J. Pienaar, and S. H. Orzack. 2008. A comparative method for studying adaptation to a randomly evolving environment. *Evolution* 62:1965-1977.
- Haynes, J. L. 1995. Standardized classification of Poeciliid development for life-history studies. *Copeia* 1995:147-154.
- 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. *J. Evol. Biol.* 19:741-754.
- Husak, J. 2006. Do female collared lizards change field use of maximal sprint speed capacity when gravid? *Oecologia* 150:339-343.
- James, R. S., and I. A. Johnston. 1998. Influence of spawning on swimming performance and muscle contractile properties in the short-horn sculpin. *J. Fish Biol.* 53:485-501.
- Kembel, S. W., P. D. Cowan, M. R. Helmus, W. K. Cornwell, H. Morlon, D. D. Ackerly, S. P. Blomberg, and C. O. Webb. 2010. Picante: R tools for integrating phylogenies and ecology. *Bioinformatics* 26:1463-1464.
- Langerhans, R. B. 2006. Evolutionary consequences of predation: avoidance, escape, reproduction, and diversification *in* A. M. T. Elewa, ed. *Predation in organisms: a distinct phenomenon*. Springer-Verlag, Heidelberg, Germany.
- Langerhans, R. B. 2008. Predictability of phenotypic differentiation across flow regimes in fishes. *Integr. Comp. Biol.* 48:750-768.

- Langerhans, R. B. 2009a. Morphology, performance, fitness: functional insight into a post-Pleistocene radiation of mosquitofish. *Biol. Lett.* 5:488-491.
- Langerhans, R. B. 2009b. Trade-off between steady and unsteady swimming underlies predator-driven divergence in *Gambusia affinis*. *J. Evol. Biol.* 22:1057-1075.
- Langerhans, R. B., and T. J. DeWitt. 2004. Shared and Unique Features of Evolutionary Diversification. *The American Naturalist* 164:335-349.
- Langerhans, R. B., M. E. Gifford, and E. O. Joseph. 2007. Ecological speciation in *Gambusia* fishes. *Evolution* 61:2056-2074.
- 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 D. N. Reznick. 2010. Ecology and evolution of swimming performance in fishes: predicting evolution with biomechanics. Pp. 200-248 in P. Domenici, and B. G. Kapoor, eds. *Fish Locomotion: an etho-ecological perspective*. Science Publishers, Enfield.
- Lauder, G. V., and E. D. Tytell. 2006. Hydrodynamics of undulatory propulsion. Pp. 425-468 in R. E. Shadwick, and G. V. Lauder, eds. *Fish Physiology*. Academic Press, San Diego.
- Lavin, S. R., W. H. Karasov, A. R. Ives, K. M. Middleton, and T. Garland Jr. 2008. Morphometrics of the avian small intestine compared with that of nonflying mammals: a phylogenetic approach. *Physiol. Biochem. Zool.* 81:526-550.

- Lighthill, M. J. 1971. Large-amplitude elongated-body theory of fish locomotion. Proceedings of the Royal Society of London. Series B. Biological Sciences 179:125-138.
- Losos, J. B. 2008. Phylogenetic niche conservatism, phylogenetic signal and the relationship between phylogenetic relatedness and ecological similarity among species. Ecol. Lett. 11:995-1003.
- Losos, J. B., and R. E. Ricklefs. 2009. Adaptation and diversification on islands. Nature 457:830-836.
- Lucinda, P. H. F. 2003. Poeciliidae. Pp. 555-581 in R. E. Reis, S. O. Kullander, and C. J. Ferraris, eds. Checklist of the Freshwater Fishes of South and Central America. EDIPUCRS, Porto Alegre, Brasil.
- Martins, E. P., and T. F. Hansen. 1997. Phylogenies and the comparative method: A general approach to incorporating phylogenetic information into the analysis of interspecific data. Am. Nat. 149:646-667.
- McGuigan, K., C. E. Franklin, C. Moritz, and M. W. Blows. 2003. Adaptation of rainbow fish to lake and stream habitats. Evolution 57:104-118.
- McHenry, M. J., and G. V. Lauder. 2006. Ontogeny of form and function: Locomotor morphology and drag in zebrafish (*Danio rerio*). J. Morphol. 267:1099-1109.
- Mitteroecker, P., and F. Bookstein. 2011. Linear discrimination, ordination, and the visualization of selection gradients in modern morphometrics. Evolutionary Biology 38:100-114.

- Monteiro, L. R. 1999. Multivariate Regression Models and Geometric Morphometrics: The Search for Causal Factors in the Analysis of Shape. *Syst. Biol.* 48:192-199.
- Oufiero, C. E., G. E. A. Gartner, S. C. Adolph, and T. Garland, Jr. 2011a. Latitudinal and climatic variation in body size and dorsal scale counts in *Sceloporus* lizards: a phylogenetic perspective. *Evolution* 65:3590-3607.
- Oufiero, C. E., M. R. Walsh, D. N. Reznick, and T. Garland, Jr. 2011b. Swimming performance trade-offs across a gradient in community composition in Trinidadian killifish (*Rivulus hartii*). *Ecology* 92:170-179.
- Pagel, M. 1999. Inferring the historical patterns of biological evolution. *Nature* 401:877-884.
- Paradis, E., J. Claude, and K. Strimmer. 2004. APE: Analyses of Phylogenetics and Evolution in R language. *Bioinformatics* 20:289-290.
- Pires, M. N., A. Banet, B. J. A. Pollux, and D. N. Reznick. 2011. Variation and evolution of reproductive strategies in J. Evans, A. Pilastro, and I. Schlupp, eds. *Ecology and Evolution of Poeciliid Fishes*. University of Chicago Press.
- 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 Part A: Ecological Genetics and Physiology* 307A:113-125.
- Plaut, I. 2002. Does pregnancy affect swimming performance of female Mosquitofish, *Gambusia affinis*? *Funct. Ecol.* 16:290-295.

- Poff, N. L., J. D. Allan, M. B. Bain, J. R. Karr, K. L. Prestegard, B. D. Richter, R. E. Sparks, and J. C. Stromberg. 1997. The Natural Flow Regime. *Bioscience* 47:769-784.
- R Development Core Team. 2011. R: A language and environment for statistical computing. R Foundation for Statistical Computing, Vienna.
- Revell, L. J., L. J. Harmon, R. B. Langerhans, and J. J. Kolbe. 2007. A phylogenetic approach to determining the importance of constraint on phenotypic evolution in the neotropical lizard *Anolis cristatellus*. *Evol. Ecol. Res.* 9:261-282.
- Rezende, E. L., and J. A. F. Diniz-Filho. 2012. Phylogenetic analyses: comparing species to infer adaptations and physiological mechanisms. *Comprehensive Physiology* 2:639-674.
- Reznick, D., R. J. Baxter, and J. Endler. 1994. Long-term studies of tropical stream fish communities: the use of field notes and museum collections to reconstruct communities of the past. *Am. Zool.* 34:452-462.
- Rohlf, F. J. 2008. tpsDig2. Distributed by the author. Department of Ecology and Evolution, State University of New York, Stony Brook.
- Rosen, D. E., and R. M. Bailey. 1963. The Poeciliid Fishes (Cyprinodontiformes), their structure, zoogeography, and systematics. *Bull. Am. Mus. Nat. Hist. N. Y.* 126:1-176.

- Sabaj-Pérez, M. H. 2010. Standard symbolic codes for institutional resource collections in herpetology and ichthyology: an Online Reference. American Society of Ichthyologists and Herpetologists, Washington D. C.
- Sabo, J. L., and D. M. Post. 2008. Quantifying periodic, stochastic, and catastrophic environmental variation. *Ecol. Monogr.* 78:19-40.
- Shine, R. 2003. Effects of pregnancy on locomotor performance: an experimental study on lizards. *Oecologia* 136:450-456.
- Stanley, S. M. 1979. *Macroevolution: Pattern and Process*. W. H. Freeman and Co., San Francisco.
- Tabachnick, B. G., and L. S. Fidell. 2001. *Using Multivariate Statistics*. Allyn & Bacon.
- Travis, J., and D. N. Reznick. 2009. Adaptation. Pp. 105-131 *in* M. Ruse, and J. Travis, eds. *Evolution: The First Four Billion Years*. Harvard University Press, Cambridge, MA.
- Van Valen, L. 1974. A natural model for the origin of some higher taxa. *J. Herpetol.* 8:109-121.
- Walker, J. A. 1997. Ecological morphology of lacustrine threespine stickleback *Gasterosteus aculeatus* L. (Gasterosteidae) body shape. *Biol. J. Linn. Soc.* 61:3-50.
- Watson, D. M. S., N. W. Timofeeff-Ressovsky, E. J. Salisbury, W. B. Turrill, T. J. Jenkin, R. R. Gates, R. A. Fisher, C. Diver, G. D. H. Carpenter, J. B. S. Haldane, E. W. MacBride, and R. N. Salaman. 1936. A discussion on the

- present state of the Theory of Natural Selection. Proceedings of the Royal Society of London. Series B - Biological Sciences 121:43-73.
- Webb, J. K., and M. J. Lannoo. 2004. Pregnancy Decreases Swimming Performance of Female Northern Death Adders (*Acanthopis praelongus*). *Copeia* 2004:357-363.
- Webb, P. W. 1982. Locomotor Patterns in the Evolution of Actinopterygian Fishes. *Am. Zool.* 22:329-342.
- Webb, P. W. 1984. Body Form, Locomotion and Foraging in Aquatic Vertebrates. *Am. Zool.* 24:107-120.
- Weeks, S. C. 1996. The Hidden Cost of Reproduction: Reduced Food Intake Caused by Spatial Constraints in the Body Cavity. *Oikos* 75:345-349.
- Wesner, J. S., E. J. Billman, A. Meier, and M. C. Belk. 2011. Morphological convergence during pregnancy among predator and nonpredator populations of the livebearing fish *Brachyrhaphis rhabdophora* (Teleostei: Poeciliidae). *Biol. J. Linn. Soc.* 104:386-392.
- Wetzel, R. G., and G. E. Likens. 2000. *Limnological analyses*. Springer Verlag, New York.
- Wohl, E., and D. M. Merritt. 2008. Reach-scale channel geometry of mountain streams. *Geomorphology* 93:168-185.
- Zandonà, E., S. K. Auer, S. S. Kilham, J. L. Howard, A. López-Sepulcre, M. P. O'Connor, R. D. Bassar, A. Osorio, C. M. Pringle, and D. N. Reznick. 2011.

Diet quality and prey selectivity correlate with life histories and predation regime in Trinidadian guppies. *Funct. Ecol.* 25:964-973.

Zelditch, M. L., D. L. Swiderski, H. D. Sheets, and W. L. Fink. 2004. *Geometric morphometrics for biologists: a primer*. Elsevier Academic Press, San Diego.

Table 1.1. Overview of collections analyzed. Predation levels: low (LP) and high (HP). Flow levels: low (LF) and high (HF). Sample size (n) for each collection correspond to the total number of individuals analyzed for estimating minimum size at maturity over the number of individuals included in shape analysis after excluding juveniles and curved individuals. Further details about about site and date of collections in Appendix 1.1.

Species	Collection	Pred.	Flow	n
<i>G. alvarezi</i>	UMMZ 211110	LP	LF	30/26
<i>G. atrora</i>	UMMZ 169499, UMMZ 210724	HP	HF	43/39
<i>G. atrora</i>	AMNH 40812, AMNH 77929	HP	HF	28/26
<i>G. aurata</i>	AMNH 75821, RBL	LP	LF	28/27
<i>G. baracoana</i>	USNM 204442	HP	LF	29/21
<i>G. clarkhubbsi</i>	TCWC 11880-09, TCWC 11882-07, TCWC 11887-08	LP	HF	34/33
<i>G. geiseri</i>	TNHC 9132, TNHC 9146	LP	LF	58/58
<i>G. hubbsi</i>	UMMZ 202727	HP	LF	21/18
<i>G. hurtadoi</i>	UMMZ 196737, UMMZ 211112	LP	HF	62/58
<i>G. krumholzi</i>	KU 7341	HP	LF	29/24
<i>G. lemaitrei</i>	UIST (uncatalogued material)	HP	LF	24/24
<i>G. luma</i>	FMNH 87628, FMNH 94163, UMMZ 197235, UMMZ 197258	HP	HF	76/52
<i>G. manni</i>	ANSP 103450, ANSP 103452, RBL	HP	LF	68/64
<i>G. melapleura</i>	USNM 205559	LP	HF	25/23
<i>G. nicaraguensis</i>	UMMZ 199657, UMMZ 199689	HP	HF	39/39
<i>G. oligosticta</i>	UMMZ 190129, RBL	HP	LF	38/36
<i>G. punctata</i>	AMNH 96308	HP	HF	20/18
<i>G. punctata</i>	AMNH 96320	HP	HF	19/18
<i>G. punctata</i>	USNM 203197, USNM 203198	HP	HF	60/54
<i>G. rhizophorae</i>	TCWC 2577-02, TCWC 8671-01, UMMZ 213650, RBL	HP	LF	36/35
<i>G. sexradiata</i>	UMMZ 210795, UMMZ 196655	HP	LF	48/47
<i>G. vittata</i>	AMNH 75838	HP	HF	20/14
<i>G. vittata</i>	UMMZ 192503	HP	HF	24/23
<i>G. vittata</i>	UMMZ 97513	HP	HF	34/31
<i>G. vittata</i>	UMMZ 97517	HP	HF	26/25
<i>G. wrayi</i>	USNM 205574, UF 25054	HP	LF	48/42

Table 1.2. MANCOVAs for the three configuration sets (shapes early in pregnancy, late in pregnancy, and the pregnancy effect). Independent variables were centroid size (CS), predation (P), flow (F), and their interaction (P*F). F-ratios were estimated from Wilk's Λ . Partial η^2 values indicate effect size. Phylogenetic MANCOVAs used Brownian motion on two phylogenetic trees, one with the original branch lengths proportional to nucleotide differences, and another with constant branch lengths. K indicates phylogenetic signal of the respective divergence vector (d). The correlations of each d with streamlining index (SI) were calculated from non-phylogenetic MANCOVAs. Significant p-values ($p < 0.05$) in bold.

Configuration	Indep. variab.	Non-phylogenetic				Original		Constant		Correlation of d with SI		
		df	F	p	Partial η^2	p	K	p	K	R ²	Slope \pm SE	p
	CS	11,11	5.069	0.006	0.86	0.001	0.354	0.012	1.098	0.05	0.75 \pm 0.67	0.274
Early in pregnancy	P	11,11	3.989	0.015	0.80	0.000	0.182	0.017	0.444	0.32	2.05 \pm 0.61	0.003
	F	11,11	1.276	0.346	0.56	0.016	0.365	0.193	0.477	0.01	0.32 \pm 0.57	0.581
	P*F	11,11	2.128	0.113	0.68	0.003	0.275	0.069	0.363	0.04	0.64 \pm 0.65	0.334
Late in pregnancy	CS	11,11	1.322	0.326	0.55	0.641	0.164	0.505	0.915	0.35	2.26 \pm 0.63	0.001
	P	11,11	3.918	0.016	0.80	0.000	0.527	0.009	0.968	0.25	1.67 \pm 0.59	0.009
	F	11,11	0.953	0.531	0.49	0.073	0.215	0.317	0.567	0.08	0.74 \pm 0.50	0.149
	P*F	11,11	1.499	0.257	0.60	0.015	0.167	0.206	0.315	0.03	-0.61 \pm 0.71	0.398
Effect of pregnancy	CS	13,9	1.181	0.411	0.56	0.735	0.071	0.559	0.624	0.18	2.19 \pm 0.97	0.033
	P	13,9	1.174	0.414	0.63	0.131	0.035	0.403	0.252	0.26	3.04 \pm 1.04	0.007
	F	13,9	1.378	0.320	0.67	0.018	0.083	0.154	0.456	0.14	2.43 \pm 1.24	0.061
	P*F	13,9	0.934	0.558	0.57	0.180	0.065	0.501	0.351	0.05	2.21 \pm 1.89	0.255

Table 1.3. Regressions showing best-supported model and average model for each dependent variable. Dependent variables were log of standard length (SL), centroid size (CS), Streamlining index (SI), Fineness Index (FI), surface area (SA) and caudal peduncle area (CP). Subscripts of dependent variables indicate estimated values early in pregnancy, late in pregnancy, and the pregnancy effect. Five adaptive models were tested for each dependent variable: M~S, M~S+P, M~S+F, M~S+P+F, M~S+P+F+P*F, where P=Predation, and F=Flow, and S= log SL. When size variables (log SL and CS) were the dependent variables, S was not used as a covariate. The models of character evolution tested were ordinary least squares (OLS, non-phylogenetic multiple regression), Pagel's (RegPagel), and Ornstein–Uhlenbeck (RegOU). Branch-length transformations were either proportional to molecular distances (M) or constant (C) branch lengths. Phylogenetic signal was λ for RegPagel, and d for RegOU. Akaike weights (w) indicate the percentage of variation explained by each model. Coefficients of the interaction (P*F) are not shown because they were invariably zero. Full list and further details of top set models available in Appendix 1.11.

Depend. variable	Independent variables	Character Evolution	Tree	w	λ or d	Coefficient \pm SE p -value			
						log SL	Predation	Flow	
log SL	P	RegOU	M	28.6	0.047		0.121 \pm 0.079	0.143	
	Average	-	-	-	-		0.077 \pm 0.080		0.014 \pm 0.045
CS	P	RegOU	M	35.8	0.109		0.217 \pm 0.114	0.074	
	Average	-	-	-	-		0.147 \pm 0.115		0.004 \pm 0.045
S _{early}	S+P	RegPagel	M	26.3	0.715		0.124 \pm 0.080	0.140	0.035
	Average	-	-	85.5	-		0.036 \pm 0.073		<0.001 \pm 0.004
S _{late}	S	RegOU	C	29.1	1.285		0.060 \pm 0.066	0.373	
	Average	-	-	73.8	-		0.028 \pm 0.056		<0.001 \pm 0.004
S _{pregnancy}	S+P	RegPagel	C	21.9	0.857		-0.020 \pm 0.066	0.763	0.025
	Average	-	-	87.3	-		-0.009 \pm 0.045		<0.001 \pm 0.004
F _{early}	S	RegOU	M	30.9	0.306		-0.026 \pm 0.088	0.775	
	Average	-	-	79.9	-		-0.017 \pm 0.075		<0.001 \pm 0.013
F _{late}	S	OLS	-	24.1	-		-0.070 \pm 0.073	0.343	
	Average	-	-	77.7	-		-0.034 \pm 0.066		0.001 \pm 0.010
F _{pregnancy}	S	OLS	-	13.7	-		0.086 \pm 0.038	0.036	
	Average	-	-	88.3	-		0.030 \pm 0.045		-0.003 \pm 0.010
S _{Aearly}	S	RegOU	M	29.3	0.487		1.941 \pm 0.082	<0.001	
	Average	-	-	84.1	-		1.220 \pm 0.790		0.005 \pm 0.019
S _{Alate}	S	RegOU	M	23.0	0.128		1.983 \pm 0.098	<0.001	
	Average	-	-	70.9	-		1.152 \pm 0.770		-0.001 \pm 0.014
S _{Apregnancy}	S	OLS	-	26.9	-		-0.068 \pm 0.030	0.035	
	Average	-	-	63.8	-		-0.031 \pm 0.034		<0.001 \pm 0.003
CP _{early}	S	RegOU	M	40.6	0.189		1.957 \pm 0.071	<0.001	
	Average	-	-	70.5	-		1.120 \pm 0.749		<0.001 \pm 0.009
CP _{late}	S	RegOU	M	23.9	0.070		2.018 \pm 0.077	<0.001	
	Average	-	-	75.8	-		1.235 \pm 0.771		-0.001 \pm 0.010

Depend. variable	Independent variables	Character Evolution	Tree	w	λ or d	Coefficient \pm SE		p-value
						log SL	Predation	
CP _{pregnancy}	S	OLS	-	28.2	-	0.034 \pm 0.057	0.559	
	Average	-	-	69.1	-	0.021 \pm 0.046	-0.003 \pm 0.012	0.001 \pm 0.006

Figure 1.1. Map with collection localities and *Gambusia* phylogeny superimposed. LP=low, and HP=high-predation regime. LF=low, and HF= high-flow regime. Geographic coordinates used WGS84 datum. Branch lengths stretched for the purpose of presentation and not directly proportional to molecular divergence. See Supplementary Material for original phylogeny.

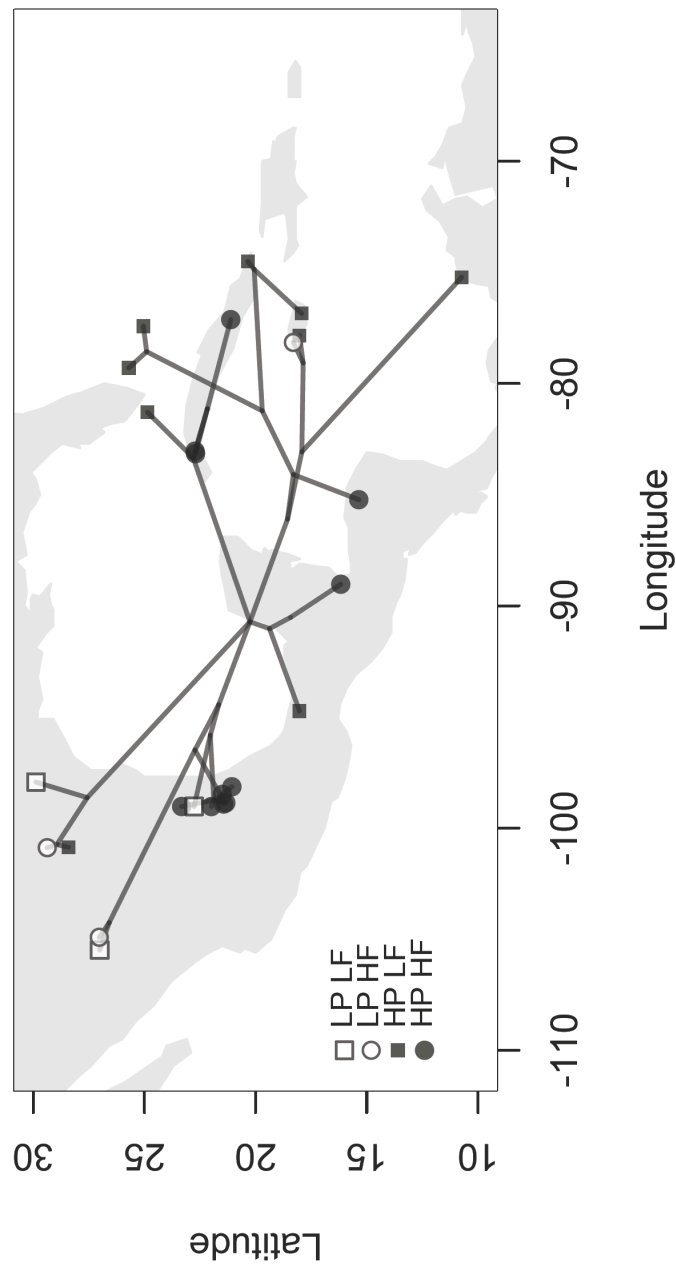


Figure 1.2. (A) Digital fish reconstruction as a composite of 200 elliptical cylinders, used for calculating streamlining index and surface area of each fish. (B) Landmarks (circles) and semilandmarks (squares) digitized for geometric-morphometric analyses. Numbers correspond to the following positions: (1) tip of the snout; (2-3) semilandmarks along dorsal midline between (1) and (4); anterior (4) and posterior (5) terminus of dorsal-fin base; dorsal (6) and ventral (7) terminus of caudal-fin base; posterior (8) and anterior (9) terminus of anal fin; (10) anterior terminus of pelvic-fin base; (11) semilandmark along ventral midline between (10) and (12); bottom of head at junction of border of opercle and body outline; and (13) eye center. Gray polygon indicates caudal peduncle area.

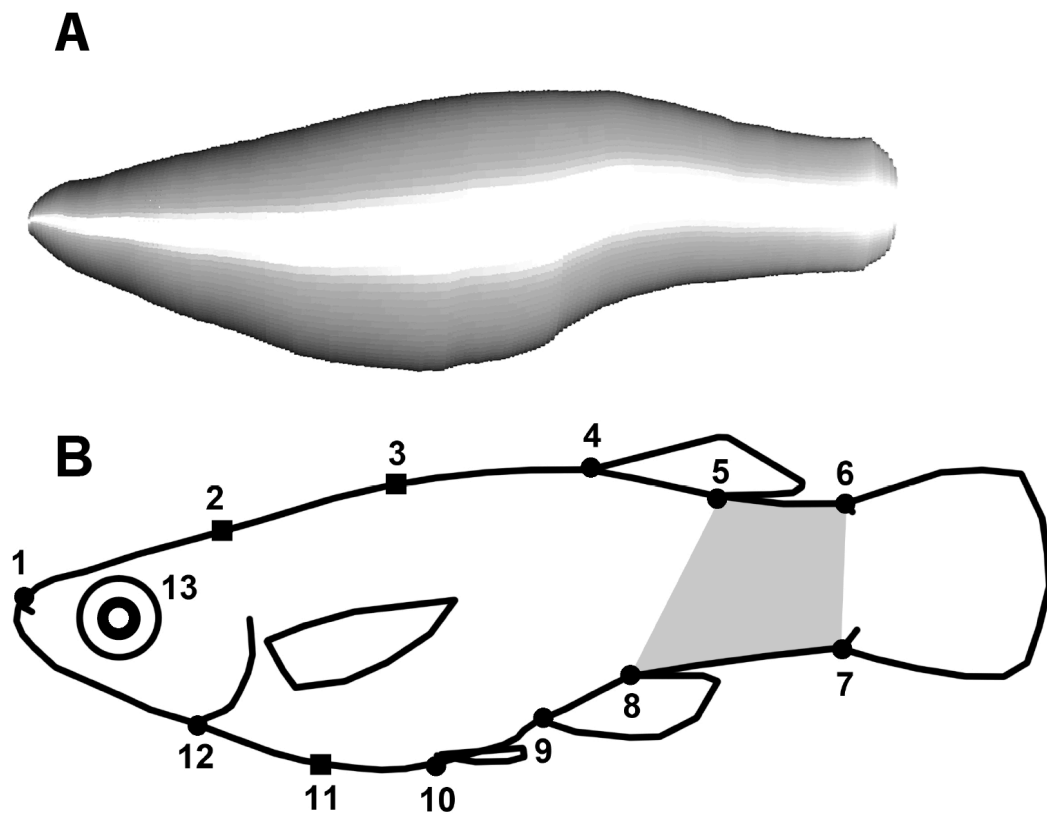


Figure 1.3. Shape variation estimated with MANCOVAs. Configuration sets in columns, independent variables in rows. Scattered and box-whisker plots show the relationship between respective divergence vectors (d) and the corresponding independent variable. Thin-plate splines show the range of shape variation, from low to high values in the independent variable values represented with grey and black outlines, respectively. Length, thickness, and darkness of arrows within shapes indicate the magnitude of change from low to high-values in the independent variable.

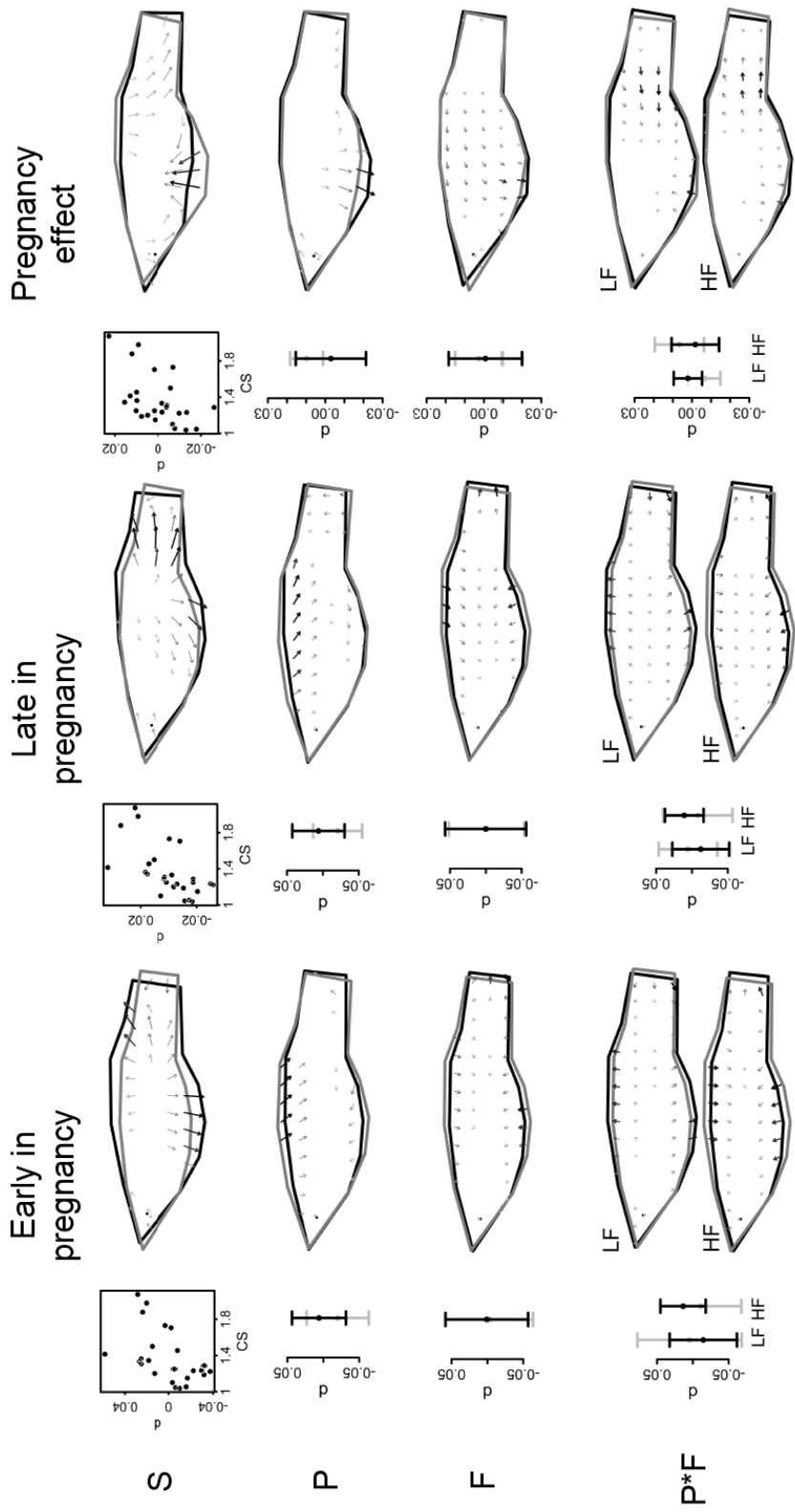
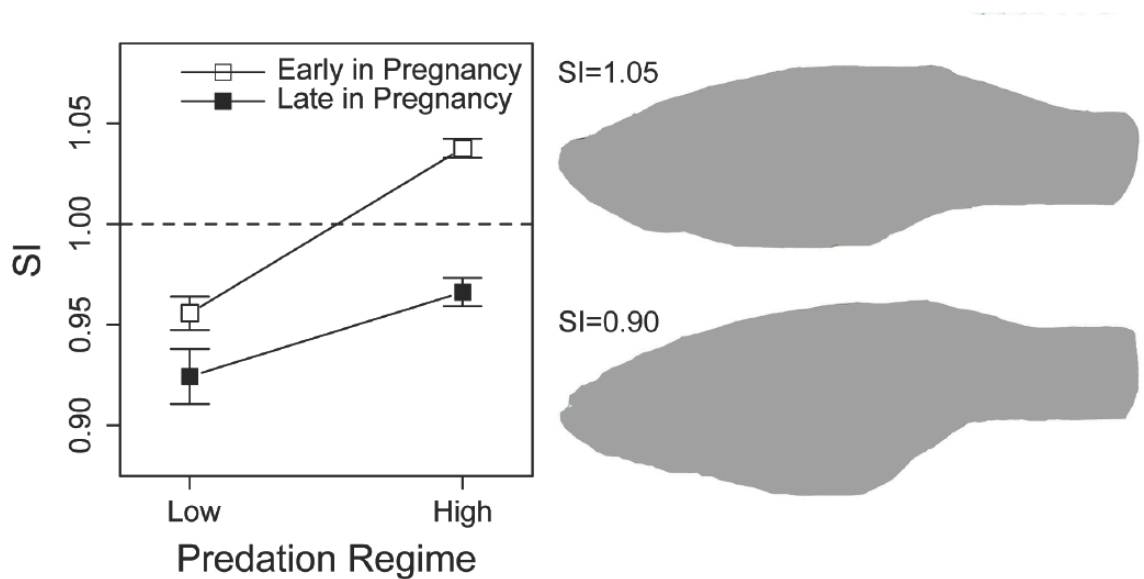


Figure 1.3

Figure 1.4. Left panel: Predicted values (mean \pm SE of estimated values) obtained from Ordinary Least Squares models showing the predation effect on streamlining index (SI) of females early and late in pregnancy. Right panel: Lateral outlines of fish with SI values at the extremes of the SI range depicted in the left panel. Horizontal dashed-line indicating optimal SI value of one. Right-side: example outlines of females across the range of SI variation.



Appendix 1.1. Additional information on collections analyzed. Geographic coordinates use WGS84 datum. Predation levels: low (LP) and high (HP). Flow levels: low (LF) and high (HF). Sample size (n) for each collection correspond to the total number of individuals analyzed for estimating minimum size at maturity over the number of individuals included in shape analysis after excluding juveniles and curved individuals.

Species	Collection	Locality	Long.	Lat.	Date	Pred.	Flow	n
<i>G. alvarezi</i>	UMMZ 211110	Ojo de San Gregorio, Chihuahua, Mexico	27.010	-105.495	22/03/82	LP	LF	30/26
<i>G. atrora</i>	UMMZ 169499	Río Axtla, San Luis Potosí, Mexico.	21.438	-98.922	31/12/51	HP	HF	24/23
<i>G. atrora</i>	UMMZ 210724	Río Huichihuayán, San Luis Potosí, Mexico.	21.438	-98.922	22/01/82	HP	HF	19/16
<i>G. atrora</i>	AMNH 40812	Río Huichihuayán, San Luis Potosí, Mexico.	21.478	-98.965	25/03/72	HP	HF	15/15
<i>G. atrora</i>	AMNH 77929	Río de la Tableta, San Luis Potosí, USA	21.520	-98.007	10/04/80	HP	HF	13/11
<i>G. aurata</i>	AMNH 75821	Río Mante, Tamaulipas, Mexico	22.699	-99.046	11/02/86	LP	LF	9/9
<i>G. aurata</i>	RBL	Río Guayalejo, Tamaulipas, Mexico.	22.832	-99.011	22/06/05	HP	LF	19/18
<i>G. baracoana</i>	USNM 204442	Lagunas de Pedro Montiel at Baracoa, Guantánamo, Cuba.	20.350	-74.507	29/12/43	HP	LF	29/21
<i>G. clarkhubbsi</i>	TCWC 11880-09	San Felipe Creek, Texas, USA.	29.371	-100.884	03/08/01	LP	HF	4/4
<i>G. clarkhubbsi</i>	TCWC 11882-07	San Felipe Creek, Texas, USA.	29.371	-100.884	23/03/02	LP	HF	16/16
<i>G. clarkhubbsi</i>	TCWC 11887-08	San Felipe Creek, Texas, USA.	29.366	-100.886	23/03/02	LP	HF	14/13
<i>G. geiseri</i>	TNHC 9132	San Marcos River, Texas, USA.	29.872	-97.931	14/12/78	LP	LF	25/25

Species	Collection	Locality	Long.	Lat.	Date	Pred.	Flow	n
<i>G. geiseri</i>	TNHC 9146	San Marcos River, Texas, USA.	29.879	-97.932	04/11/78	LP	LF	33/33
<i>G. hubbsi</i>	UMMZ 202727	Massey Creek, Bimini, Bahamas.	25.700	-79.297	01/08/51	HP	LF	21/18
<i>G. hurtadoi</i>	UMMZ 196737	Ojo Hacienda Dolores, Chihuahua, Mexico.	27.030	-104.915	27/03/74	LP	HF	28/28
<i>G. hurtadoi</i>	UMMZ 211112	Ojo Hacienda Dolores, Chihuahua, Mexico.	27.030	-104.915	28/03/82	LP	HF	34/30
<i>G. krumholzi</i>	KU 7341	Río de Nava, Coahuila, Mexico.	28.412	-100.868	14/04/63	HP	LF	29/24
<i>G. lemaitrei</i>	UIST (uncataloged)	Ciénaga Totumo, Atlántico, Colombia.	10.734	-75.222	02/12/06	HP	LF	24/24
<i>G. luma</i>	FMNH 87628	Belize River, Cayo, Belize.	17.117	-89.128	24/04/76	HP	HF	35/24
<i>G. luma</i>	FMNH 94163	Blue Creek, Toledo, Belize.	16.202	-89.036	14/07/71	HP	HF	24/17
<i>G. luma</i>	UMMZ 197235	Río Paujila, Izabal, Guatemala.	15.686	-88.981	12/04/74	HP	HF	13/8
<i>G. luma</i>	UMMZ 197258	Río Dulce, Izabal, Guatemala.	15.670	-88.984	13/04/74	HP	HF	4/3
<i>G. manni</i>	ANSP 103450	Great Bahama Bank, New Providence Island, Bahamas.	25.060	-77.437	09/04/55	HP	LF	30/27
<i>G. manni</i>	ANSP103452	Lake Killarney, New Providence Island, Bahamas.	25.040	-77.416	15/04/55	HP	LF	21/20
<i>G. manni</i>	RBL	Lake Killarney, New Providence, Bahamas.	25.040	-77.416	27/07/05	HP	LF	17/17
<i>G. melapleura</i>	USNM 205559	Shrewsbury River, Westmoreland, Jamaica.	18.300	-78.153	29/12/49	LP	HF	25/23
<i>G. nicaraguensis</i>	UMMZ 199657	Río Coco, Zelaya, Nicaragua.	14.745	-83.976	15/05/75	HP	HF	21/21
<i>G. nicaraguensis</i>	UMMZ 199689	Stream at West side of Big Hog Island, Honduras.	15.971	-86.479	20/05/75	HP	HF	18/18
<i>G. oligosticta</i>	RBL	Pool near Port Royal, Kingston, Jamaica.	17.936	-76.806	30/06/05	HP	LF	13/13

Species	Collection	Locality	Long.	Lat.	Date	Pred.	Flow	n
<i>G. oligosticta</i>	UMMZ 190129	Port Henderson, St Catherine, Jamaica.	17.923	-76.893	12/12/70	HP	LF	25/23
<i>G. punctata</i>	AMNH 96308	Río Taco Taco, Pinar del Río, Cuba.	22.699	-83.158	17/04/90	HP	HF	20/18
<i>G. punctata</i>	AMNH 96320	Río San Cristóbal, Pinar del Río, Cuba.	22.721	-83.042	17/04/90	HP	HF	19/18
<i>G. punctata</i>	USNM 203197	Street 25 mi W of Camagüey, Camagüey, Cuba.	21.445	-78.146	20/06/48	HP	HF	25/21
<i>G. punctata</i>	USNM 203198	Río Gibara, Oriente, Cuba.	20.788	-76.103	28/07/44	HP	HF	35/33
<i>G. rhizophorae</i>	RBL	Tidal creek in Matheson Hammock County Park, Florida, USA.	25.679	-80.262	13/07/05	HP	LF	8/8
<i>G. rhizophorae</i>	TCWC 2577-02	Key West, Florida, USA.	24.564	-81.774	09/08/78	HP	LF	4/4
<i>G. rhizophorae</i>	TCWC 8671-01	Salt pond at Key West, Florida, USA.	24.558	-81.768	18/03/97	HP	LF	6/5
<i>G. rhizophorae</i>	UMMZ 213650	Monroe Lagoon at Hopkins Island, Florida, USA.	24.629	-81.380	30/12/85	HP	LF	18/18
<i>G. sexradiata</i>	UMMZ 196655	Laguna at Rancho Chapultepec, Tabasco, Mexico.	17.922	-93.363	10/03/74	HP	LF	20/19
<i>G. sexradiata</i>	UMMZ 210795	Isolated lagoons close to Papalopán, Oaxaca, Mexico.	18.127	-96.106	26/01/82	HP	LF	28/28
<i>G. vittata</i>	AMNH 75838	Río Tancuilín, San Luis Potosí, Mexico.	21.338	-98.867	28/02/86	HP	HF	20/14
<i>G. vittata</i>	UMMZ 192503	Río Guayalejo at Llera, Tamaulipas, Mexico.	23.324	-99.032	09/06/69	HP	HF	24/23
<i>G. vittata</i>	UMMZ 97513	Río Valles, San Luis Potosí, Mexico.	21.989	-99.034	28/04/30	HP	HF	34/31

Species	Collection	Locality	Long.	Lat.	Date	Pred. Flow	n
<i>G. vittata</i>	UMMZ 97517	Tributary of Río Calabozo, Veracruz, Mexico.	21.064	-98.143	07/05/30	HP HF	26/25
<i>G. wrayi</i>	UF 25054	Black River Spa Spring, St Elizabeth, Jamaica.	18.026	-77.844	18/04/78	HP LF	18/14
<i>G. wrayi</i>	USNM 205574	Black River Spa Spring, St Elizabeth, Jamaica.	18.023	-77.847	28/12/49	HP LF	30/28

Appendix 1.2. Fish community of each locality included in analyses. Numbers in Fish Community correspond to the Species Number of Appendix 1.3.

Species	Collection	Fish Community
<i>G. alvarezi</i>	UMMZ 211110	34, 89
<i>G. atrora</i>	AMNH 40812	7, 35, 56, 69, 103, 124, 128
<i>G. atrora</i>	AMNH 77929	7, 35, 56, 69, 103, 124, 128
<i>G. atrora</i>	UMMZ 169499	1, 6, 20, 35, 54, 56, 58, 65, 66, 67, 70, 88, 90, 103, 106, 107, 112, 113, 114, 127, 128
<i>G. atrora</i>	UMMZ 210724	1, 6, 20, 35, 54, 56, 58, 65, 66, 67, 70, 88, 90, 103, 106, 107, 112, 113, 114, 127, 128
<i>G. aurata</i>	AMNH 75821	75, 102, 103, 112
<i>G. aurata</i>	RBL	6, 36, 49, 56, 65, 66, 90, 100, 103, 112, 113
<i>G. baracoana</i>	USNM 204442	37, 84
<i>G. clarkhubbsi</i>	TCWC 11880-09	21, 25, 32, 38, 55
<i>G. clarkhubbsi</i>	TCWC 11882-07	21, 25, 32, 38, 55
<i>G. clarkhubbsi</i>	TCWC 11887-08	21, 25, 32, 38, 55
<i>G. geiseri</i>	TNHC 9132	31, 39, 78
<i>G. geiseri</i>	TNHC 9146	31, 39, 78
<i>G. hurtadoi</i>	UMMZ 196737	23, 41
<i>G. hurtadoi</i>	UMMZ 211112	23, 41
<i>G. krumholzi</i>	KU 7341	2, 33, 42, 65, 79
<i>G. lemaitrei</i>	UIST (uncatalogued material)	43, 71, 98, 99, 110
<i>G. luma</i>	FMNH 87628	4, 6, 9, 11, 16, 18, 44, 68, 96, 118, 122
<i>G. luma</i>	FMNH 94163	6, 10, 12, 44, 44, 63, 68, 68, 108, 117, 125, 125

Species	Collection	Fish Community
<i>G. luma</i>	UMMZ 197235	4, 5, 6, 9, 11, 13, 14, 15, 16, 18, 19, 26, 29, 44, 63, 64, 73, 80, 93, 95, 103, 108, 109, 116, 119, 125
<i>G. luma</i>	UMMZ 197258	4, 5, 6, 9, 13, 16, 18, 44, 63, 83, 91, 95, 119
<i>G. manni</i>	ANSP 103450	22, 40, 45, 85
<i>G. manni</i>	ANSP 103452	22, 40, 45, 85
<i>G. manni</i>	RBL	22, 40, 45, 85
<i>G. hubbsi</i>	UMMZ 202727	45, 86
<i>G. melapleura</i>	USNM 205559	46
<i>G. nicaraguensis</i>	UMMZ 199657	3, 6, 9, 11, 47, 74, 92, 95, 97, 101, 111
<i>G. nicaraguensis</i>	UMMZ 199689	47, 84, 104
<i>G. oligosticta</i>	RBL	48, 86
<i>G. oligosticta</i>	UMMZ 190129	24, 48, 84
<i>G. punctata</i>	AMNH 96308	50, 61, 84
<i>G. punctata</i>	AMNH 96320	50, 51, 59, 60, 62, 82, 84, 87
<i>G. punctata</i>	USNM 203197	50, 84
<i>G. punctata</i>	USNM 203198	50, 61, 84
<i>G. rhizophorae</i>	RBL	52, 86
<i>G. rhizophorae</i>	TCWC 2577-02	52, 86
<i>G. rhizophorae</i>	TCWC 8671-01	52, 86
<i>G. rhizophorae</i>	UMMZ 213650	52, 86
<i>G. sexradiata</i>	UMMZ 196655	4, 6, 8, 16, 17, 28, 53, 72, 96, 103, 105, 121, 122, 123, 126

Species	Collection	Fish Community
<i>G. sexradiata</i>	UMMZ 210795	6, 11, 16, 27, 53, 72, 94, 103, 106, 108, 109, 120, 126
<i>G. vittata</i>	AMNH 75838	6, 35, 49, 56, 65, 66, 69, 76, 103, 106, 113, 115, 124, 127, 128, 129
<i>G. vittata</i>	UMMZ 192503	6, 20, 28, 56, 65, 66, 77, 100, 102, 103, 112, 113, 129
<i>G. vittata</i>	UMMZ 97513	6, 20, 49, 56, 65, 66, 76, 77, 112, 113, 129
<i>G. vittata</i>	UMMZ 97517	6, 49, 56, 65, 66, 127
<i>G. wrayi</i>	UF 25054	30, 57, 81, 84
<i>G. wrayi</i>	USNM 205574	30, 57, 81, 84

Appendix 1.3. Diet of fish inhabiting localities of this study. Species numbers match numbers in Appendix 1.2. Synonym was the name in field notes if it is different from the current valid name. Predation indicates whether the species never eats other fish (low-predation, LP), rarely eats fish (middle-predation, MP), or it is feeds mainly on fish (high-predation, HP). Food items per species listed in descending order of importance. Abbreviations with references correspond to method used to estimate feeding habits. FE, field experiment; ISO, isotope analysis; LE, laboratory experiment; LR, literature review; ND, no data; NS, not specified; OBS, direct observation; PO, All Poeciliids except for *Belonesox belizanus* were assumed not to predate strongly on *Gambusia* (Bussing, 1998; Meffe and Snelson, 1989); SCA, stomach content analysis; XR, X-ray observations.

Species Number	Species	Synonym	Pred.	Food items and comments
1	<i>Agonostomus monticola</i>	NA	MP	Invertebrates, algae, detritus(Aiken, 1998: SCA). Mostly insects, also algae and occasional fish (Cruz, 1987:SCA). Mostly insects and insect larvae, also plant material, and probably algae (Hildebrand, 1935: SCA). Plant material, invertebrates, rarely fish (Phillip, 1993: SCA). Invertebrates, algae, rarely plant material or fish (Torres-Navarro and Lyons, 1999: SCA).

Species Number	Species	Synonym	Pred.	Food items and comments
2	<i>Ameiurus natalis</i>	NA	HP	Detritus, fishes, plant material and invertebrates (Walters et al., 2003: LR). Trophic level 2.9-3.6 in a scale where 2 is herbivore, 3 carnivore, and 4-4.3 is piscivore (Fry et al., 1999: ISO). Ate fish when used as bait (Schneider, 1998: FE). Larvae of other fish (Carpenter and Mueller, 2008: OBS). Population in the Everglades of Florida, USA, is mainly predatory (Murie et al., 2009: LR).
3	<i>Amphilophus margaritifer</i>	<i>Cichlasoma margaritiferum</i>	NA	ND
4	<i>Amphilophus robertsoni</i>	<i>Cichlasoma robertsoni</i>	LP	Mostly insects, zooplankton, and molluscs (Valtierra-Vega and Schmitter-Soto, 2000: SCA). Algae and invertebrates by sifting mud (Conkel, 1993: OBS).
5	<i>Anchoa parva</i>	NA	LP	Plankton (Miller, 2005: LR)
6	<i>Astyanax fasciatus</i>	NA	MP	Algae, invertebrates, and fish material (Esteves, 1996: SCA). ostly invertebrates and plant material (Vilella, et al., 2002: SCA).
7	<i>Astyanax mexicanus</i>	NA	HP	Plant material, invertebrates, detritus, fish eggs, larvae and juveniles (Miller, 2005: LR). Omnivorous but important predator of swordtails (Xiphophorus, Poeciliidae) (Rosenthal, et al., 2001: LE).

Species Number	Species	Synonym	Pred.	Food items and comments
8	<i>Atherinella alvarezi</i>	NA	NA ND	
9	<i>Atherinella sp.</i>	NA	NA	ND. Some species of <i>Atherinella</i> eat insects and fruits (Miller, 2005: LR).
10	<i>Awaous tajasica</i>	NA	LP	Aquatic insects, foraminifera, and plant material (Miller, 2005: LR).
11	<i>Belonesox belizanus</i>	NA	HP	Fish and insects (Miller, 2005: LR). Fish (Bussing, 1998: LR). Fish (Meffe and Snelson, 1989: OBS).
12	<i>Brycon guatemalensis</i>	NA	MP	Insects when young, switching to plant material herbivorous as adults. A population in Lago Arenal (Costa Rica) also feeds heavily on fishes (silversides, Atherinidae) (Bussing, 1998: LR).
13	<i>Carlhubbsia stuarti</i>	NA	LP PO	
14	<i>Centropomus ensiferus</i>	NA	MP	Crustaceans, rarely in fish (Alvarez-Lajonchère and Tsuzuki. 2008: LR).
15	<i>Centropomus undecimalis</i>	NA	HP	Mainly fish (including <i>Gambusia</i>), also shrimp, polychaetes, amphipods, and calenoid copepods (Luczkovich et al., 1995: SCA, OBS).

Species Number	Species	Synonym	Pred.	Food items and comments
16	<i>Cichlasoma salvini</i>	NA	MP	Opportunistic omnivore with tendency to herbivory, although some populations are zooplanktivorous (Miller, 2005: LR). Macroinvertebrates and some small fish (Conkel, 1993: OBS). Mostly aquatic invertebrates and vegetative detritus (Cochran, 2008: SCA).
17	<i>Cichlasoma urophthalmum</i>	<i>Cichlasoma urophthalmus</i>	MP	Mainly fish, also detritus, ostracods, algae, snails, and terrestrial insects (Bergmann and Motta, 2002: SCA). Plant material and invertebrates (Chávez-López et al., 2004: SCA). Invertebrates, vegetal material, and in less extent fish (Martínez-Palacios and Ross, 1988: OBS, SCA,).
18	<i>Cryptoheros spilurus</i>	<i>Cichlasoma spilurum</i>	LP	Mostly algae and invertebrates (Valtierra-Vega and Schmitter-Soto, 2000: SCA).
19	<i>Cryptoheros spilurus</i>	<i>Cichlasoma spinosissimum</i>	LP	Mostly algae and invertebrates (Valtierra-Vega and Schmitter-Soto, 2000: SCA).
20	<i>Cyprinella lutrensis</i>	<i>Notropis lutrensis</i>	LP	Detritus, invertebrates, and algae, rarely on fish scales (Gido et al 1999: SCA).
21	<i>Cyprinella proserpina</i>	NA	LP	Invertebrates (Thomas et al., 2007: LR). Invertebrates (Bonner et al., 2008: LR).
22	<i>Cyprinodon laciniatus</i>	NA	LP	Plant matter and detritus (Barton, 1999: OBS).

Species Number	Species	Synonym	Pred.	Food items and comments
23	<i>Cyprinodon macrolepis</i>	NA	LP	ND. <i>Cyprinodon macrolepis</i> was assumed to be non-predatory of <i>Gambusia hurtadoi</i> (a sympatric species) because it is a small fish that does not exceed 44 mm in SL (Miller et al., 2005), and its congeners are typically detritivorous and invertivorous (Miller et al., 2005).
24	<i>Cyprinodon variegatus</i>	NA	LP	Plant material, mosquito larvae, pelecypods, copepods, and shrimp (Miller, 2005: LR).
25	<i>Dionda diaboli</i>	NA	LP	In captivity, live brine shrimp or flaked or fry food (Thomas et al., 2007: LR). Algae (López-Fernández and Winemiller, 2005: LR).
26	<i>Dormitator maculatus</i>	NA	MP	Plant material, immature insects, algae, Ostracoda, Copepoda, and Cladocera (Nordlie, 1981: SCA). Plant material and detritus, in less extent invertebrates and fish (Teixeira, 1994: SCA).
27	<i>Dorosoma anale</i>	NA	LP	Filter feeder (Froese, et al., 2011: LR).
28	<i>Dorosoma petenense</i>	NA	LP	Plankton (Miller, 2005: LR). Phytoplankton and brine shrimp (Holanov and Tash, 1978: LE).
29	<i>Eleotris amblyopsis</i>	NA	MP	Insect and shrimp larvae, other invertebrates, in less extent plant material and fish (Nordlie, 1981: SCA). Fish and crustaceans (Bussing, 1998: LR). Plant material, invertebrates, and other fishes (Miller, 2005: LR).

Species Number	Species	Synonym	Pred.	Food items and comments
30	<i>Eleotris pisonis</i>	NA	HP	Plant material, fish, and invertebrates (Nordlie, 1981: SCA). Molluscs, crustaceans, and fish (Teixeira, 1994: SCA). Crustaceans, fish, and molluscs Winemiller and Ponwith 1998: SCA). Insects and fish (Brian Langerhans, pers. obs.: SCA).
31	<i>Etheostoma fonticola</i>	NA	LP	Inmature insects and microcrustacea (Thomas et al., 2007: LR).
32	<i>Etheostoma grahami</i>	NA	LP	ND. <i>Etheostoma grahami</i> was assumed to be non-predatory of <i>Gambusia geiseri</i> (a sympatric species) because it is a small fish that does not exceed 60 mm in total length (Thomas et al., 2007), and its congeners consume invertebrates and microcrustacea (e.g. Paine et al., 1982).
33	<i>Gambusia affinis</i>	NA	LP PO	
34	<i>Gambusia alvarezi</i>	NA	LP PO	
35	<i>Gambusia atrora</i>	NA	LP PO	

Species Number	Species	Synonym	Pred.	Food items and comments
36	<i>Gambusia aurata</i>	NA	LP PO	
37	<i>Gambusia baracoana</i>	NA	LP PO	
38	<i>Gambusia clarkhubbsi</i>	NA	LP PO	
39	<i>Gambusia geiseri</i>	NA	LP PO	
40	<i>Gambusia hubbsi</i>	NA	LP PO	
41	<i>Gambusia hurtadoi</i>	NA	LP PO	

Species Number	Species	Synonym	Pred.	Food items and comments
42	<i>Gambusia krumhozi</i>	NA	LP PO	
43	<i>Gambusia lemaitrei</i>	NA	LP PO	
44	<i>Gambusia luma</i>	NA	LP PO	
45	<i>Gambusia manni</i>	NA	LP PO	
46	<i>Gambusia melapleura</i>	NA	LP PO	
47	<i>Gambusia nicaraguensis</i>	NA	LP PO	

Species Number	Species	Synonym	Pred.	Food items and comments
48	<i>Gambusia oligosticta</i>	NA	LP PO	
49	<i>Gambusia panuco</i>	NA	LP PO	
50	<i>Gambusia punctata</i>	NA	LP PO	
51	<i>Gambusia puncticulata</i>	NA	LP PO	
52	<i>Gambusia rhizophorae</i>	NA	LP PO	
53	<i>Gambusia sexradiata</i>	NA	LP PO	

Species Number	Species	Synonym	Pred.	Food items and comments
54	<i>Gambusia sp.</i>	NA	LP PO	
55	<i>Gambusia speciosa</i>	NA	LP PO	
56	<i>Gambusia vittata</i>	NA	LP PO	
57	<i>Gambusia wrayi</i>	NA	LP PO	
58	<i>Girardinichthys viviparus</i>	<i>Girardinichthys innominatus</i>	LP	Omnivorous with a tendency to carnivory (Armando Vega-López, per. com.). Microcrustacea (Vega-López et al., 2007: LE). The maximum size of this species (65 mm SL, Miller, 1965) indicates that it is unlikely to be a dangerous predator for <i>Gambusia</i> .
59	<i>Girardinus falcatus</i>	NA	LP PO	

Species Number	Species	Synonym	Pred.	Food items and comments
60	<i>Girardinus metallicus</i>	NA	LP PO	
61	<i>Girardinus sp.</i>	NA	LP PO	
62	<i>Girardinus uninotatus</i>	NA	LP PO	
63	<i>Gobiomorus dormitor</i>	<i>Gobiomorus dormitor</i>	HP	Shrimp larvae and fish (Nordlie, 1981 : SCA). Fish (Miller, 2005: LR). Fish (B. Langerhans, unpub.).
64	<i>Gobiosoma sp.</i>	NA	NA	ND. Some species of <i>Gobiosoma</i> eat insects and fish (Miller, 2005: LR).
65	<i>Herichthys cyanoguttata</i>	<i>Cichlasoma cyanoguttatum</i>	HP	Fish (Brosnan et al., 2003: LE).
66	<i>Herichthys labridens</i>	<i>Cichlasoma labridens</i>	MP	Mostly snails, rarely fish (Rosenthal et al., 2001 : SCA).

Species Number	Species	Synonym	Pred.	Food items and comments
67	<i>Herichthys steindachneri</i> <i>i</i>	<i>Cichlasoma steindachneri</i>	HP	Fish (Miller, 2005: LR).
68	<i>Heterandria bimaculata</i>	NA	LP PO	
69	<i>Heterandria jonesi</i>	NA	LP PO	
70	<i>Heterandria sp.</i>	NA	LP PO	
71	<i>Hoplias malabaricus</i>	NA	HP	Fish (Dahl, 1971: LR, OBS).
72	<i>Hyphessobrycon compressus</i>	NA	MP	Omnivore, with an intermediate trophic level (Winemiller et al., 2011: ISO).

Species Number	Species	Synonym	Pred.	Food items and comments
73	<i>Hyphessobrycon milleri</i>	NA	NA ND	
74	<i>Hyphessobrycon tortugerae</i>	NA	NA ND	
75	<i>Ictiobus bubalus</i>	NA	LP	Detritus, insects and microinvertebrates (Gido, 2001: SCA). Ostracods, insect larvae, snails, algae, and detritus (Miller, 2005: LR).
76	<i>Ictiobus labiosus</i>	NA	NA ND	
77	<i>Lepisosteus osseus</i>	NA	HP	Fish, rarely on invertebrates (Lagler and Hubbs, 1940: SCA).
78	<i>Lepomis macrochirus</i>	NA	LP	Zooplankton and invertebrates (Olson et al., 2003: SCA).

Species Number	Species	Synonym	Pred.	Food items and comments
79	<i>Lepomis megalotis</i>	NA	MP	Insects, other small invertebrates, and occasionally fish (Miller, 2005: LR).
80	<i>Leptophilypnus fluvialtilis</i>	NA	MP	Invertebrates (Winemiller and Leslie, 1992: LR, OBS).
81	<i>Limia melanogaster</i>	NA	LP PO	
82	<i>Limia vittata</i>	NA	LP PO	
83	<i>Lophogobius cyprinoides</i>	NA	LP	Algae, secondarily on amphipods, isopods, copepods, tanaids, ostracods, insects, polychaetes, mollusks, and barnacles (Darcy, 1981: SCA).
84	<i>Lowland-Marine</i>	NA	HP	Marine predators can ascend freshwater streams. Most of lowland streams in the Neotropics have a number of piscivore fishes (D. Reznick, pers. obs).
85	<i>Lutjanus griseus</i>	NA	HP	Shrimp, fish, and crabs (Harrigan et al., 1989: SCA, ISO).

Species Number	Species	Synonym	Pred.	Food items and comments
86	<i>Marine</i>	NA	HP	Predators such as needlefish and barracudas are ubiquitous in marine habitats inhabited by <i>Gambusia</i> .
87	<i>Nandopsis tetracanthus</i>	<i>Cichlasoma tetracanthus</i>	MP	Worms, small fish, shrimps, and water insects (Froese et al., 2011: LR). <i>Poecilia reticulata</i> (as lure) (Hulsey et al., 2010: LE).
88	<i>Neoheteran dria tridentiger</i>	NA	LP PO	
89	<i>Notropis chihuahua</i>	<i>Notropis sp.</i>	LP	Invertebrates (Burr and Mayden, 1981: SCA). Invertebrates (De La Maza-Benignos, 2009: NS).
90	<i>Notropis tropicus</i>	NA	NA ND	
91	<i>Oligopites palometa</i>	NA	MP	Fish scales, benthic and planktonic crustaceans, rarely smaller fishes and polychaetes (Froese et al., 2011: LR).

Species Number	Species	Synonym	Pred.	Food items and comments
92	<i>Parachromis managuensis</i>	<i>Cichlasoma managuense</i>	HP	Fish (Bussing, 1998: LR).
93	<i>Parachromis motaguensis</i>	<i>Cichlasoma motaguense</i>	MP	Invertebrates and fish (Froese et al., 2011: LR).
94	<i>Paraneotroplus fenestratus</i>	<i>Cichlasoma fenestratum</i>	LP	Mainly vegetable material and invertebrates (Froese et al., 2011: LR).
95	<i>Paraneotroplus maculicauda</i>	<i>Cichlasoma maculicauda</i>	LP	Detritus and vegetable matter (Froese et al., 2011: LR).
96	<i>Petenia splendida</i>	NA	HP	Fish (Miller, 2005: LR).
97	<i>Phallichthys amates</i>	NA	LP PO	
98	<i>Poecilia caucana</i>	NA	LP PO	
99	<i>Poecilia cuneata</i>	NA	LP PO	

Species Number	Species	Synonym	Pred.	Food items and comments
100	<i>Poecilia formosa</i>	NA	LP PO	
101	<i>Poecilia gilli</i>	NA	LP PO	
102	<i>Poecilia latipunctata</i>	NA	LP PO	
103	<i>Poecilia mexicana</i>	NA	LP PO	
104	<i>Poecilia orri</i>	NA	LP PO	
105	<i>Poecilia petenensis</i>	NA	LP PO	
106	<i>Poecilia sphenops</i>	NA	LP PO	

Species Number	Species	Synonym	Pred.	Food items and comments
107	<i>Poeciliidae</i> <i>sp.</i>	NA	NA ND	
108	<i>Rhamdia</i> <i>guatemalensis</i>	NA	MP	Aquatic insects, crustaceans, and small fishes (Bussing, 1998: LR). Considered a predator of the Poeciliidae Brachirhaphis rhabdophora (Langerhans and DeWitt, 2004: LR).
109	<i>Rocio</i> <i>octofasciata</i>	<i>Cichlasoma</i> <i>octofasciatum</i>	MP	Invertebrates and algae (Valtierra-Vega and Schmitter-Soto, 2000: SCA). Worms, crustaceans, insects and fish (Froese et al., 2011: LR).
110	<i>Roebooides</i> <i>dayi</i>	NA	LP	Invertebrates and fish scales (Peterson and Winemiller, 1997: SCA).
111	<i>Roebooides</i> <i>guatemalensis</i>	NA	NA ND	
112	<i>Tampichthys</i> <i>erimyzonops</i>	<i>Dionda</i> <i>erimyzonops</i>	LP	Filamentous algae and detritus (Miller, 2005: LR).

Species Number	Species	Synonym	Pred.	Food items and comments
113	<i>Tampichthys ipni</i>	<i>Dionda ipni</i>	LP	Insects, water mites, algae, and detritus (Miller, 2005: LR).
114	<i>Tampichthys rasconis</i>	<i>Dionda rasconis</i>	NA	ND
115	<i>Tampichthys sp.</i>	<i>Dionda sp.</i>	LP	ND. However, the only species of <i>Tampichthys</i> in this region of Rio Panuco are <i>T. ipni</i> and <i>T. erimyzonops</i> , both non-piscivorous species (Miller, 2005).
116	<i>Theraps bocourti</i>	<i>Cichlasoma bocourti</i>	NA	ND
117	<i>Theraps godmani</i>	<i>Cichlasoma godmani</i>	LP	Fresh vegetation (Froese et al., 2011: LR).
118	<i>Theraps intermedium</i>	<i>Cichlasoma intermedium</i>	NA	ND
119	<i>Thorichthys aureus</i>	<i>Cichlasoma aureum</i>	NA	ND

Species Number	Species	Synonym	Pred.	Food items and comments
120	<i>Thorichthys ellioti</i>	<i>Cichlasoma ellioti</i>	NA ND	
121	<i>Thorichthys helleri</i>	<i>Cichlasoma helleri</i>	NA ND	
122	<i>Thorichthys meeki</i>	<i>Cichlasoma meeki</i>	MP	Invertebrates, algae, and rarely fish (Valtierra-Vega and Schmitter-Soto, 2000: SCA).
123	<i>Thorichthys pasionis</i>	<i>Cichlasoma pasionis</i>	NA	ND. This species shows molariform teeth on their pharyngeal jaws, a character associated with a molluscivore diet (Chakravarty, 2007).
124	<i>Xiphophorus cortezi</i>	NA	LP PO	
125	<i>Xiphophorus helleri</i>	NA	LP	Invertebrates, algae, rarely fish scales (Arthington, 1989: SCA).

Species Number	Species	Synonym	Pred.	Food items and comments
126	<i>Xiphophorus maculatus</i>	NA	LP	Invertebrates and algae (Arthington, 1989: SCA).
127	<i>Xiphophorus montezumae</i>	NA	LP PO	
128	<i>Xiphophorus pygmaeus</i>	NA	LP PO	
129	<i>Xiphophorus variatus</i>	NA	LP PO	

Note: There is no data available on the diet of some species sympatric with the *Gambusia* analyzed (NAs in Predation column). This lack of knowledge was not a problem to determine the predation regime of the localities where these species lived because their localities had piscivorous fish species, making them HP sites.

References in Appendix 1.3

Aiken, K. A. 1998. Reproduction, diet and population structure of the mountain mullet, *Agonostomus monticola*, in Jamaica, West Indies. *Environmental Biology of Fishes* 53: 347-352.

Alvarez-Lajonchère, L. and M. Tsuzuki. 2008. A review of methods for *Centropomus* spp. (snooks) aquaculture and recommendations for the establishment of their culture in Latin America. *Aquaculture Research* 39, 684-700.

Arthington, A. H. 1989. Diet of *Gambusia affinis holbrooki*, *Xiphophorus helleri*, *X. maculatus* and *Poecilia reticulata* (Pisces: Poeciliidae) in streams of Southeastern Queensland, Australia. *Asian Fisheries Science* 2: 193-212.

Barton, M. 1999. Threatened fishes of the world: *Cyprinodon laciniatus* Hubbs & Miller, 1942 (Cyprinodontidae). *Environmental Biology of Fishes* 55: 422.

Bergmann, G. T., and P. J. Motta. 2005. Diet and morphology through ontogeny of the nonindigenous Mayan cichlid '*Cichlasoma (Nandopsis)*' *urophthalmus* (Günther 1862) in southern Florida. *Environmental Biology of Fishes* 72: 205-211.

Bierbach, D., M. Schulte, N. Herrmann, M. Tobler, S. Stadler, C. T Jung, B. Kunkel, R. Riesch, S. Klaus, M. Ziege, J. Rimber Indy, L. Arias-Rodriguez, and M. Plath. 2010. Predator-induced changes of female mating preferences: innate and experiential effects. *BMC Evolutionary Biology* 2011, 11:190.

Bonner, T., H., J. M. Watson, and C. S. Williams. 2008. Threatened fishes of the world: *Cyprinella proserpina* Girard, 1857 (Cyprinidae). *Environmental Biology of Fishes*.81:365–366.

Brosnan, S. F., R. L. Earley, and L. A. Dugatkin. 2003. Observational Learning and Predator Inspection in Guppies (*Poecilia reticulata*). *Ethology* 109, 823-833.

Burr, B- M. and R. L. Mayden. 1981. Systematics, Distribution and Life History Notes on *Notropis chihuahua* (Pisces: Cyprinidae). *Copeia* 1981: 255-265.

Bussing, W. A. 2008. *Astyanax cocibolca*, a new characid (Pisces: Ostariophysi) from Lake Nicaragua, Central America. *Revista de Biología Tropical* 56: 1361-1370.

Bussing, W. A. 1998. Peces de las aguas continentales de Costa Rica. *Revista de Biología Tropical* 46 (Suppl. 2): 1-468.

Carpenter, J., and G. A. Mueller. 2008. Small nonnative fishes as predators of larval razorback suckers. *The Southwestern Naturalist* 53: 3236-242.

Chakrabarty, P. 2007. A morphological phylogenetic analysis of Middle American cichlids with special emphasis on the section '*Nandopsis*' sensu Regan.

Miscellaneous Publications, Museum of Zoology, University of Michigan No. 198: i-iv + 1-31.

Chávez-López, R., M. S. Peterson, N. J. Brown-Peterson, A. A. Morales-Gómez, and J. Franco-López. 2005. Ecology of the Mayan Cichlid, *Cichlasoma urophthalmus* Günther, in the Alvarado Lagoonal system, Veracruz, Mexico. *Gulf and Caribbean Research* Vol 16(2): 1-10. 2005

Cochran, J. 2008. Diet, habitat, and ecomorphology of cichlids in the upper Bladen River, Belize. MSc Thesis. Texas A&M University.

Conkel, D. 1993. Cichlids of North & Central America. *Cichlids North & Central America*. 1993: 1-191.

Cruz, G. A. 1987. Reproductive biology and feeding habits of cuyamel, *Joturus pichardi* and tepemechin, *Agonostomus monticola* (Pisces; Mugilidae) from Rio Platano, Mosquitia, Honduras. Bulletin of Marine Science 40: 63-72.

Dahl G. 1971. Los Peces del Norte de Colombia. Ministerio de Agricultura, Instituto de Desarrollo de los Recursos Naturales Renovables (Inderena). Talleres Litografia Arco. Bogota, 391pp.

Darcy, G. H. 1981. Food habits of the crested goby, *Lophogobius cyprinoides*, in Two Dade County, Florida, Waterways. Bulletin of Marine Science. 31: 928-932.

De la Maza Benignos, M., (editor). 2009. Los Peces del Río Conchos. Alianza WWF - FGRA y Gobierno del Estado de Chihuahua.

Derrick P. A. and V. S. Kennedy. 1997. Prey selection by the hogchoker, *Trinectes maculatus* (Pisces: Soleidae), along summer salinity gradients in Chesapeake Bay, USA. Marine Biology 129: 699-711.

Fowler, H. W. 1906. Some cold-blooded vertebrates of the Florida Keys. Proceedings of the Academy of Natural Sciences of Philadelphia 58: 77-113.

Froese, R. and D. Pauly. Editors. 2011. FishBase. World Wide Web electronic publication. www.fishbase.org, version (06/2011).

Fry, B., P. L. Mumford, F. Tam, D. D. Fox, G. L. Warren, K. E. Havens, and A. D. Steinman. 1999. Trophic position and individual feeding histories of fish from Lake Okeechobee, Florida. *Canadian Journal of Fisheries and Aquatic Sciences* 56: 590-600.

Gido, K. B. 2001. Feeding Ecology of Three Omnivorous Fishes in Lake Texoma (Oklahoma-Texas). *The Southwestern Naturalist* 46: 23-33.

Gido, K. B., J. F. Schaefer, K. Work, P. W. Lienesch, E. Marsh- Matthews, and W. J. Matthews. 1999. Effects of Red Shiner (*Cyprinella lutrensis*) on Red River Pupfish (*Cyprinodon rubrofluviatilis*). *The Southwestern Naturalist* 44: 287-295.

Harrigan, P., J. C. Zieman and S. A. Macko. 1989. The base of nutritional support for the gray snapper (*Lutjanus griseus*): an evaluation based on a combined stomach content and stable isotope analysis. *Bulletin of Marine Science* 44: 65-77.

Hildebrand, S. F. 1935. An annotated list of fishes of the fresh waters of Puerto Rico. *Copeia* 1935: 49-56.

Holanov, S. H., and J. C. Tash. 1978. Particulate and filter feeding in threadfin shad, *Dorosoma petenense*, at different light intensities. *Journal of Fish Biology* 13: 619-625.

Horstkotte, J. and M. Plath. 2008. Divergent evolution of feeding substrate preferences in a phylogenetically young species flock of pupfish (*Cyprinodon* spp.). *Naturwissenschaften* 95:1175-1180.

Hulsey, C. D., P. R. Hollingsworth Jr., Holzman, R. 2010. Co-evolution of the premaxilla and jaw protrusion in cichlid fishes (Heroine: Cichlidae). *Biological Journal of the Linnean Society* 100: 619-629.

Lagler, K. F. and F. V. Hubbs. 1940. Food of the Long-Nosed Gar (*Lepisosteus osseus oxyurus*) and the Bowfin (*Amia calva*) in Southern Michigan. *Copeia* 1940: 239-241.

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

López-Fernández and K. O. Winemiller. 2005. Status of *Dionda diaboli* and report of established populations of extic fish species in Lower San Felipe Creek, Val Verde County, Texas. *The Southwestern Naturalist*, 50: 246-251.

Luczkovich, J. J., S. F. Norton, and R. G. Gilmore, Jr . 1995. The influence of oral anatomy on prey selection during the ontogeny of two percoid fishes, *Lagodon rhomboides* and *Centropomus undecimalis*. *Environmental Biology of Fishes* 44: 79-95.

Meffe, G. K. and F. F. Snelson. 1989. An ecological overview of Poeciliid fishes. In: Meffe, G. K. and F. F. Snelson (eds). *Ecology and Evolution of Livebearing Fishes*. Prentice Hall.

Miller, R. R. 2005. *Freshwater Fishes of México*. University of Chicago Press.

Murie, D. J., D. C. Parkyn, W. F. Loftus, and L. G. Nico. 2009. Variable growth and longevity of yellow bullhead (*Ameiurus natalis*) in the Everglades of south Florida, USA. *Journal of Applied Ichthyology* 25: 740–745.

Nordlie, F. G. 1981. Feeding and reproductive biology of eleotrid fishes in a tropical estuary. *Journal of Fish Biology* 18: 97-110.

Olson, N. W., C. P. Paukert, D. W. Willis, and J. A. Klammer. 2003. Prey selection and diets of bluegill *Lepomis macrochirus* with differing population characteristics in two Nebraska natural lakes. *Fisheries Management and Ecology* 10, 31–40.

Paine, M. D., J. J. Dodson, and G. Power. 1982. Habitat and food resource partitioning among four species of darters (Percidae: *Etheostoma*) in a southern Ontario stream. *Canadian Journal of Zoology*. 60: 1635-1641.

Peterson, C. C. and K. O. Winemiller. 1997. Ontogenic diet shifts and scale-eating in *Roebooides dayi*, a Neotropical characid. *Environmental Biology of Fishes* 49: 111–118.

Phillip, D. A. T. 1998. Biodiversity of freshwater fishes of Trinidad and Tobago, West Indies. PhD Thesis, University of St. Andrews.

Rosenthal, G. G., T. Y. Flores Martinez, F. J. García de León, and M. J. Ryan. 2001. Shared preferences by predators and females for male ornaments in swordtails. *American Naturalist* 158:146-154.

Schneider, J. C. 1998. Fate of dead fish in a small lake. *American Midland Naturalist* 140: 192-196.

- Stevenson, M. M. 1992. Food Habits within the Laguna Chichancanab
Cyprinodon (Pisces: Cyprinodontidae) Species Flock. The Southwestern
Naturalist 37: 337-343.
- Strecker, U. 2005. Description of a new species from Laguna Chichancanab,
Yucatan, Mexico: *Cyprinodon suavius* (Pisces: Cyprinodontidae). *Hydrobiologia*
541: 107-115.
- Taylor, D. S. 1992. Diet of the killifish *Rivulus marmoratus* collected from land
crab burrows, with further ecological notes. *Environmental Biology of Fishes* 33:
389-393.
- Tee-Van, J. 1935. Cichlid fishes in the West Indies with especial reference to
Haiti, including the description of a new species of *Cichlasoma*. *Zoologica*,
Scientific Contributions of the New York Zoological Society 10: 281-300.
- Teixeira, R. L. 1994. Abundance, reproductive period, and feeding habits of
eleotrid fishes in estuarine habitats of north-east Brazil. *Journal of Fish Biology*
45: 749-761.
- Thomas, C., T. H. Bonner, and B. G. Whiteside. 2007. *Freshwater Fishes of
Texas: A Field Guide*. Texas A&M Press.

Torres-Navarro, C. I., and Lyons, J. 1999. Diet of *Agonostomus monticola* (Pisces: Mugilidae) in the Río Ayuquila, Sierra de Manantlán Biosphere Reserve, México. *Revista de Biología Tropical* 47: 1087-1092.

Yañez-Arancibia, A., A. L. Lara-Dominguez. 1988. Ecology of three sea catfishes (Ariidae) in a tropical coastal ecosystem - Southern Gulf of Mexico. *Marine Ecology Progress Series* 49:215-230.

Vega-López, A., L. Martínez-Tabche, M. Galar Martínez. 2007. Toxic effects of waterborne polychlorinated biphenyls and sex differences in an endangered goodeid fish (*Girardinichthys viviparus*). *Environment International* 33: 540-545.

Vilella, F. S., F. G. Becker, and S. M. Hartz. 2002. Diet of *Astyanax* species (Teleostei, Characidae) in an Atlantic Forest River in Southern Brazil. *Brazilian Archives of Biology and Technology* 45: 223 - 232.

Walters, D. M., D. S. Leigh, M. C. Freeman, B. J. Freeman, and C. M. Pringle. 2003. Geomorphology and fish assemblages in a Piedmont river basin, U.S.A. *Freshwater Biology* 48, 1950–1970.

Winemiller, K. O. and M. A. Leslie. 1992. Fish assemblages across a complex, tropical freshwater/marine ecotone. *Environmental Biology of Fishes* 34 : 29-50.

Winemiller, K. O. and B. J. Ponwith. 1998. Comparative ecology of electroid fishes in Central American coastal streams. *Environmental Biology of Fishes* 53: 373-384.

Winemiller, K. O., D. J. Hoeinghaus, A. A. Pease, P. C. Esselman, R. L. Honeycutt, D. Gbanaador, E. Carrera, and J. Payne. 2011. Stable isotope analysis reveals food web structure and watershed impacts along the fluvial gradient of a mesoamerican coastal river. *River Research Applications* 27:791-803.

Appendix 1.4. Calculation of streamlining

Streamlining Index (SI) is based on a comparison of the distributions of the masses of a fish and a streamlined foil (McHenry and Lauder 2006). To calculate distributions of mass, the volumes of the fish and the foil were approximated as composites of multiple slices (elliptical cylinders, Figure 2A). For the fish, the dimensions of the elliptical cylinders were obtained by measuring the height and width from lateral and ventral pictures, respectively, at equidistant positions along the longitudinal axis. Fins were digitally removed from pictures before analyses using Photoshop CS5 (Adobe Systems Inc.). In our study we made 200 sections because exactitude does not increase with more sections (McHenry and Lauder 2006). For the ideal shape the widths and heights of the sections are estimated using the equation for NACA foils with no chamber (Jacobs et al. 1933):

$$h_{NACA}(x) = 0.4h_{\max} \left(0.2969\sqrt{\frac{x}{SL}} - 0.1260\frac{x}{SL} - 0.3516\left(\frac{x}{SL}\right)^2 + 0.2843\left(\frac{x}{SL}\right)^3 - 0.1015\left(\frac{x}{SL}\right)^4 \right)$$

where h_{NACA} is the estimated width (or height) at a particular position in the x -axis (i.e. the anterior-posterior axis), h_{\max} is the maximum width (or height) in the profile, x is the distance between the tip of the fish and the current position along the x -axis, and SL is the standard length. The distributions of mass of either the actual fish volume or the ideal volume are estimated as the moments of inertia of their respective cylinders. The total moment of inertia for each body is the sum of

the moments of inertia of its cylinders, according to the parallel axis theorem (Meriam and Kraige 2006). The moment of inertia is calculated as if the body were rotating along a vertical axis located at its anterior tip, with the equation

$$I = \frac{\rho\pi}{4} \left(\sum_{i=1}^n \left(\frac{w^3 h}{16} + whx^2 \right)_i \right) \Delta x$$

where I is the moment of inertia, ρ is density (assumed as 1 in this study), w and h are respectively the width and height of the fish at the position x of the i th section, n the number of sections, and Δx is the distance between sections (SL/200 in this study). SI is the ratio between the moments of inertia of the fish and its respective estimated NACA foil, $SI = I_{\text{fish}}/I_{\text{NACA}}$.

Fineness Index (FI), is calculated as:

$$FI = 1 - \left| 1 - \frac{SL}{4.5 D_{\max}} \right|$$

where SL is the standard length and D_{\max} is the maximum body depth. The value 4.5 comes from the experimental observation that at this ratio the drag coefficient is minimum (Blake 1983). The Matlab code used to calculate streamlining (streamline_analysis_20120312_for_distribution.m) is available upon request.

References Appendix 1.4

Blake, R. W. 1983. Fish locomotion. Cambridge University Press, Cambridge.

Jacobs, E. N., K. E. Ward, and R. M. Pinkerton. 1933. The characteristics of 78 related airfoil sections from tests in the variable-density wind tunnel. NACA Report 460.

McHenry, M. J., and G. V. Lauder. 2006. Ontogeny of form and function: Locomotor morphology and drag in zebrafish (*Danio rerio*). *J. Morphol.* 267:1099-1109.

Meriam, J. L., and L. G. Kraige. 2006. Engineering Mechanics. Vol. 2, Dynamics. John Wiley & Sons, New York.

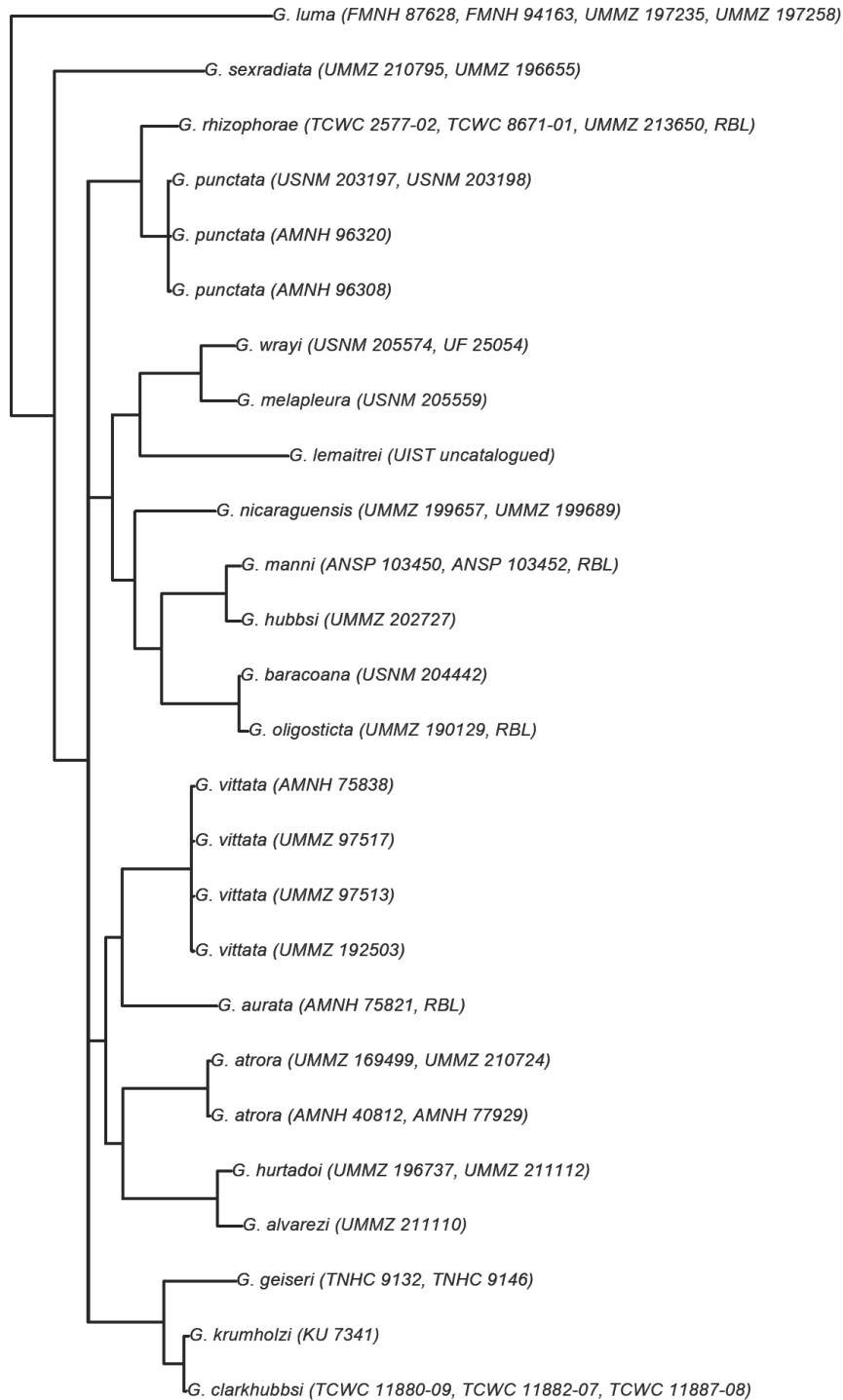
Appendix 1.5. Phylogeny of *Gambusia*

Majority (50%) consensus tree of *Gambusia* using three genes (ND2, Cyt b, S7) in Newick format (R. B. Langerhans, unpub.). Tip labels correspond to species and collection names linked by underscores, and node labels to bootstrapping support.

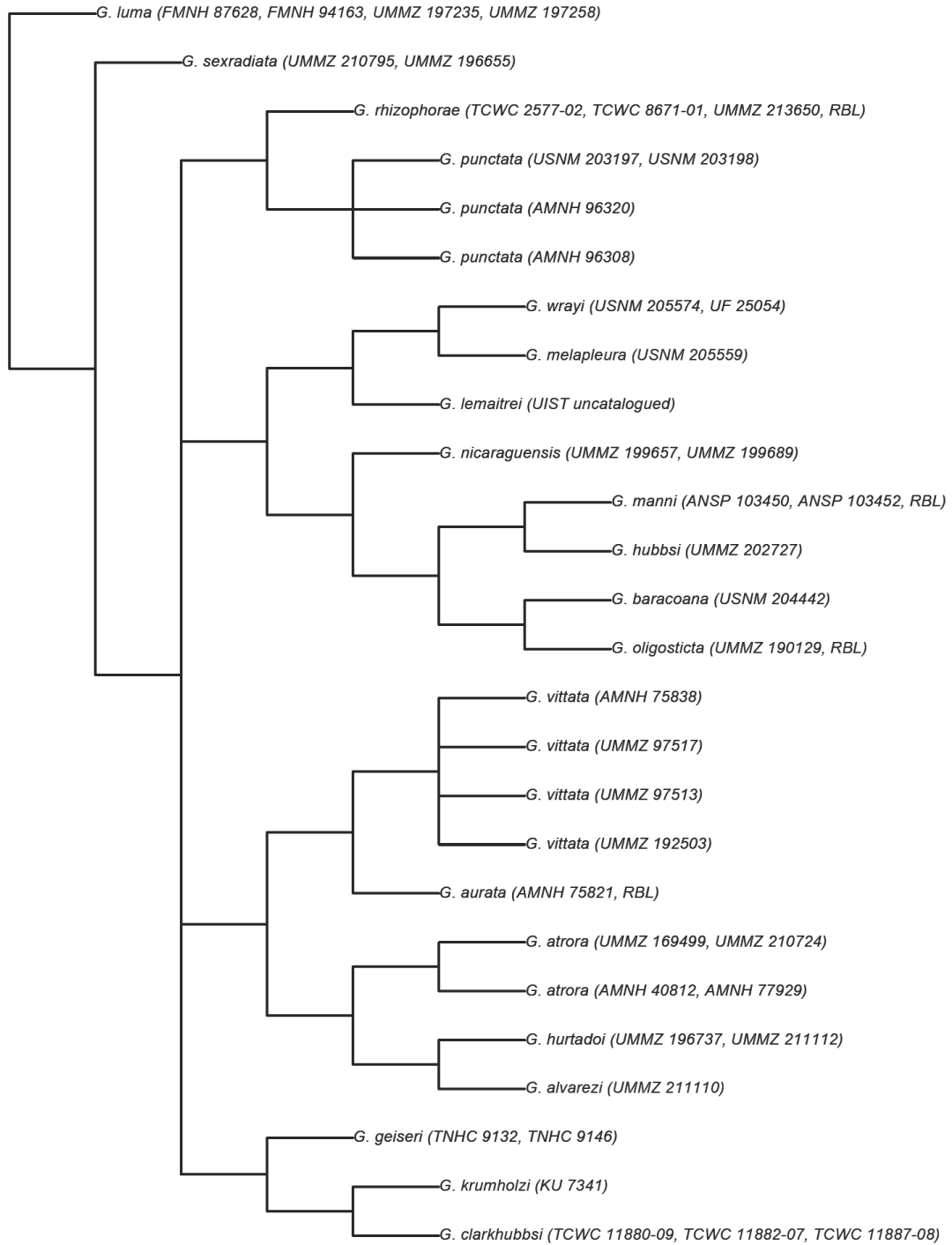
```
((((G._clarkhubbsi_-  
TCWC_11880-09_TCWC_11882-07_TCWC_11887-08:0.004177,G._krumholzi_-  
KU_7341:0.007942)1.00:0.035231,G._geiseri_-  
TNHC_9132_TNHC_9146:0.12442)0.99:0.130828,(((G._alvarezi_-  
UMMZ_211110:0.042105,G._hurtadoi_-  
UMMZ_196737_UMMZ_211112:0.023829)1.00:0.164551,(G._atrora_-  
AMNH_40812_AMNH_77929:0.00461,G._atrora_-  
UMMZ_169499_UMMZ_210724:0.00461):0.146954)0.75:0.029413,(G._aurata_-  
AMNH_75821_RBL:0.164195,(G._vittata_-UMMZ_192503:0.00461,G._vittata_-  
UMMZ_97513:0.00461,G._vittata_-UMMZ_97517:0.00461,G._vittata_-  
AMNH_75838:0.00461):0.120073)0.96:0.028746)1.00:0.029974,  
(((G._oligosticta_-UMMZ_190129_RBL:0.015952,G._baracoana_-  
USNM_204442:0.002092)1.00:0.133733,(G._hubbsi_-  
UMMZ_202727:0.023658,G._manni_-ANSP_103450_ANSP_103452_RBL:  
0.02423)0.66:0.112589)1.00:0.04566,G._nicaraguensis_-
```

UMMZ_199657_UMMZ_199689:0.139127)1.00:0.039811,(G._lemaitrei_-
UIST_uncatalogued:0.256976,(G._melapleura_-
USNM_205559:0.060682,G._wrayi_-USNM_205574_UF_25054:0.058121)
1.00:0.105819)1.00:0.04822)0.67:0.041355,((G._punctata_-
AMNH_96308:0.00461,G._punctata_-AMNH_96320:0.00461,G._punctata_-
USNM_203197_USNM_203198:0.00461):0.045904,G._rhizophorae_-
TCWC_2577-02_TCWC_8671-01_UMMZ_213650_RBL:0.062711)
0.99:0.092292)1.00:0.058663,G._sexradiata_-
UMMZ_210795_UMMZ_196655:0.258672)0.59:0.07495,G._luma_-
FMNH_87628_FMNH_94163_UMMZ_197235_UMMZ_197258:0.451326)1.00;

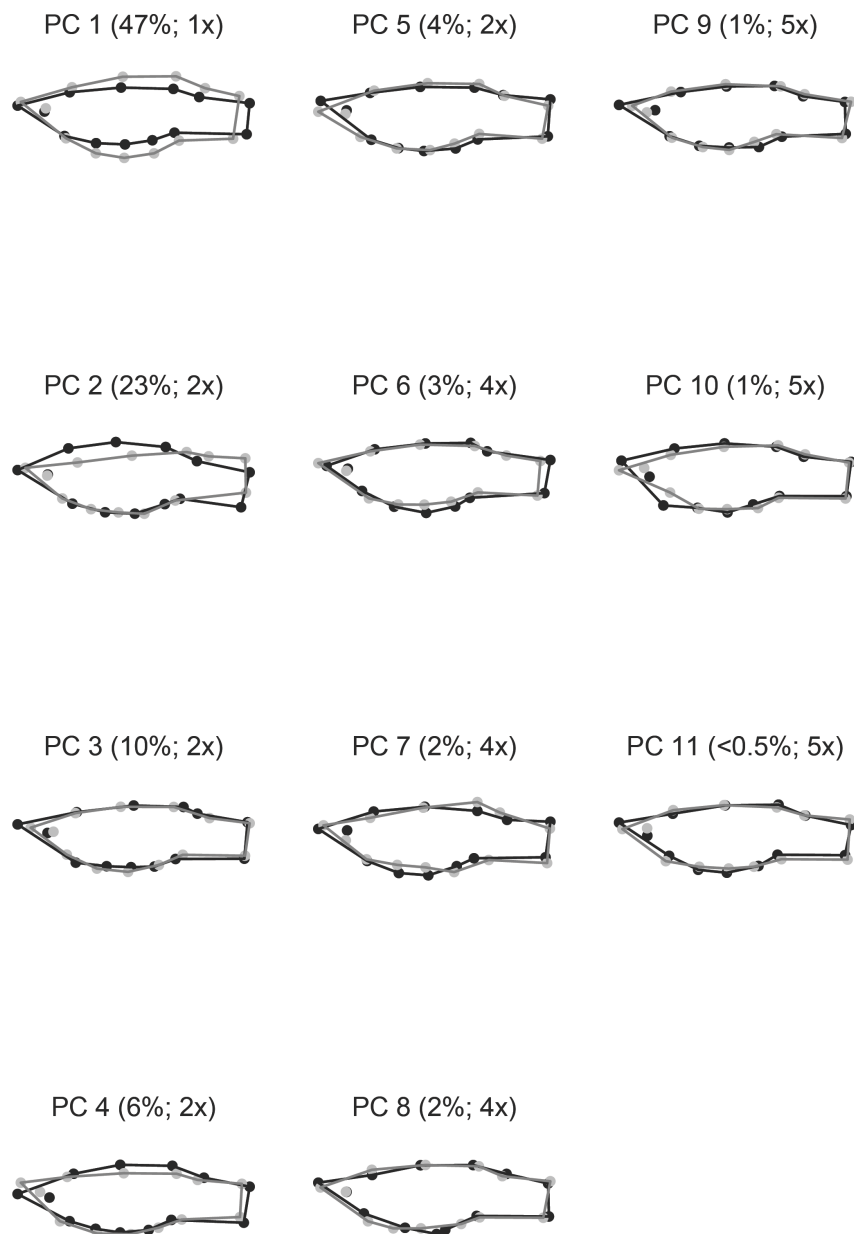
Appendix 1.6. Phylogenetic tree of *Gambusia* with branch lengths proportional to the units of inferred nucleotide substitution.



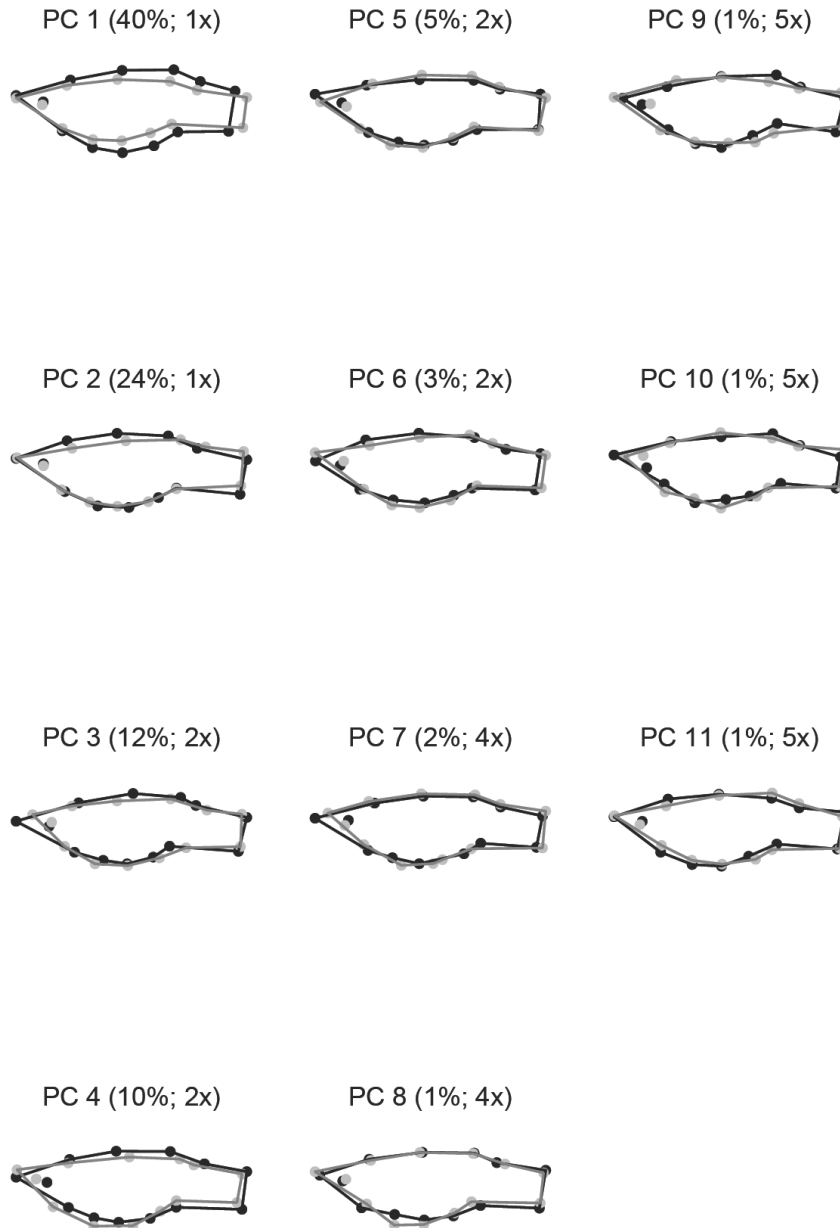
Appendix 1.7. Phylogenetic tree of *Gambusia* with constant branch lengths.



Appendix 1.8. Shape variation of females early in pregnancy, as described by Principal Components (PC). Overlapping figures represent the extreme configurations in each PC, from lowest (grey outline) to highest values (black outline). Within parentheses: Percentage of shape variation explained for each PC, and factor of magnification of differences.



Appendix 1.9. Shape variation of females late in maturity. Further details in caption of Appendix 1.8.



Appendix 1.10. Difference in shape between females early and late in pregnancy, with shape variation arranged in Principal Components. Overlapping figures are the deformations related to pregnancy on the average-shape female. Further details in caption of Appendix 1.8.

PC 1 (29%; 1x)



PC 2 (18%; 2x)



PC 3 (13%; 2x)



PC 4 (11%; 2x)



PC 5 (8%; 2x)



PC 6 (6%; 4x)



PC 7 (4%; 4x)



PC 8 (3%; 4x)



PC 9 (2%; 5x)



PC 10 (2%; 5x)



PC 11 (1%; 5x)



PC 12 (1%; 6x)



PC 13 (1%; 6x)



PC 14 (<0.5%; 6x)



Appendix 1.11. Candidate set ($\Delta AIC_c=4$) of regressions for size variables and each morphological trait. Subscripts of morphological dependent variables indicate estimated values early in pregnancy, late in pregnancy, and pregnancy effect. Five adaptive models were tested for each dependent variable: M~S, M~S+P, M~S+F, M~S+P+F, M~S+P+F+P*F, where S=log SL, P=Predation, and F=Flow. The same models without S as the covariate were tested with body size variables (log SL, Centroid Size) as dependent variables. Branch-length transformations were either proportional to molecular distances (M) or constant (C) branch lengths. Phylogenetic signal was λ for RegPagel, and d for RegOU. k =number of parameters, $\ln ML$ =log Maximal Likelihood, AIC_c =small sample corrected Akaike Information Criterion, and w =Akaike weights. Evidence Ratio (ER) indicates the number of times the most supported model of the set is better supported than each row's model. Coefficients of the interaction (P*F) are not shown because they were invariably zero.

Model	Character Evolution	Tree	k	lnML	AIC _c	w	λ or d	ER	Coefficients \pm SE				
									log SL	Predation	Flow		
Body size													
P	RegOU	M	4	12.2	-14.6	28.6	0.047	1.00		0.121 \pm 0.079	0.143		
P	RegOU	C	4	11.5	-13.1	13.9	0.698	0.49		0.116 \pm 0.075	0.142		
F	RegOU	M	4	11.2	-12.6	10.5	0.014	0.37				0.029 \pm 0.074	0.701
P	RegPagel	C	4	11.2	-12.5	10.1	0.983	0.35		0.125 \pm 0.074	0.107		
P+F	RegOU	M	5	12.4	-11.8	7.1	0.036	0.25		0.126 \pm 0.081	0.142	0.037 \pm 0.075	0.631
F	RegOU	C	4	10.7	-11.6	6.4	0.565	0.22				0.047 \pm 0.065	0.484

Model	Character Evolution	Tree	k	lnML	AICc	w	λ or d	ER	Coefficients \pm SE			
									log SL	Predation	Flow	
F	RegPagel	C	4	10.4	-10.9	4.7	0.781	0.16			0.062 \pm 0.063	0.341
P+F	RegOU	C	5	11.9	-10.8	4.3	0.655	0.15	0.119 \pm 0.076	0.138	0.051 \pm 0.063	0.432
P	RegOU	M	4	2.9	4.2	35.8	0.109	1.00	0.217 \pm 0.114	0.074		
P	RegOU	C	4	1.9	6.0	13.9	0.835	0.39	0.186 \pm 0.107	0.100		
P	RegPagel	C	4	1.8	6.2	12.7	1.045	0.35	0.201 \pm 0.104	0.070		
P+F	RegOU	M	5	2.9	7.1	8.1	0.100	0.23	0.222 \pm 0.120	0.083	0.030 \pm 0.111	0.792
F	RegOU	M	4	1.4	7.2	7.8	0.036	0.22			0.016 \pm 0.110	0.886
Streamlining Index												
S+P	RegPagel	M	5	34.6	-56.2	23.6	0.700	1.00	0.124 \pm 0.080	0.140	0.091 \pm 0.035	0.021
S+P	OLS	-	4	32.5	-55.0	13.5	-	1.75	0.109 \pm 0.081	0.195	0.072 \pm 0.035	0.053
S+P	RegPagel	C	5	33.8	-54.6	11.1	0.900	2.13	0.055 \pm 0.087	0.535	0.102 \pm 0.033	0.007
S+P	RegOU	C	5	33.6	-54.3	9.3	0.700	2.56	0.073 \pm 0.089	0.429	0.098 \pm 0.035	0.012
S+P	RegOU	M	5	33.5	-53.9	7.8	0.200	3.03	0.145 \pm 0.091	0.129	0.105 \pm 0.038	0.015
S	RegPagel	M	4	31.7	-53.5	6.3	0.600	3.70	0.183 \pm 0.083	0.041		
S	OLS	-	3	30.3	-53.5	6.2	-	3.85	0.144 \pm 0.084	0.103		
S+P+F	RegPagel	M	6	34.6	-52.7	4.3	0.700	5.56	0.126 \pm 0.083	0.151	0.090 \pm 0.037	0.029
S	RegOU	C	4	38.4	-66.9	29.1	1.285	1.00	0.060 \pm 0.066	0.373		
S	RegPagel	C	4	37.8	-65.6	15.1	1.045	1.92	0.052 \pm 0.069	0.461		
S+P	RegOU	C	5	39.1	-65.2	12.5	1.287	2.33	0.032 \pm 0.070	0.657	0.030 \pm 0.026	0.276
S	RegPagel	M	4	37.3	-64.7	9.4	0.696	3.13	0.105 \pm 0.066	0.129		
S+F	RegOU	C	5	38.5	-64.0	6.6	1.282	4.35	0.058 \pm 0.067	0.400		0.006 \pm 0.020
S+P	RegPagel	C	5	38.5	-63.9	6.5	0.989	4.55	0.028 \pm 0.073	0.711	0.031 \pm 0.028	0.276
S+P	RegPagel	C	5	41.1	-69.1	21.9	0.857	1.00	-0.020 \pm 0.066	0.763	-0.067 \pm 0.025	0.018
S+P	RegOU	C	5	40.7	-68.4	15.5	0.802	1.41	-0.038 \pm 0.067	0.580	-0.067 \pm 0.026	0.021
S	OLS	-	3	37.0	-67.0	7.4	-	2.94	0.015 \pm 0.065	0.825		
S+P	OLS	-	4	38.3	-66.7	6.6	-	3.33	0.036 \pm 0.065	0.587	-0.043 \pm 0.028	0.137
S	RegOU	M	4	38.3	-66.7	6.5	0.002	3.33	-0.046 \pm 0.071	0.526		
S	RegOU	C	4	38.0	-66.2	5.0	0.549	4.35	-0.072 \pm 0.071	0.324		

Model	Character Evolution	Tree	k	lnML	AICc	w	λ or d	ER	Coefficients \pm SE					
									log SL	Predation	Flow			
S	RegPagel	C	4	38.0	-66.2	5.0	0.658	4.35	-0.049 \pm 0.069	0.489				
S+P	RegOU	M	5	39.5	-66.1	4.8	0.006	4.55	-0.029 \pm 0.072	0.692	-0.045 \pm 0.029	0.135		
S+P	RegPagel	M	5	39.5	-66.0	4.5	0.669	4.76	-0.029 \pm 0.066	0.668	-0.068 \pm 0.029	0.034		
S+P+F	RegPagel	C	6	41.1	-65.8	4.3	0.865	5.00	-0.026 \pm 0.069	0.709	-0.066 \pm 0.026	0.023	0.008 \pm 0.020	0.703
S+P+F	RegOU	C	6	40.8	-65.1	3.0	0.807	7.14	-0.042 \pm 0.070	0.554	-0.066 \pm 0.027	0.027	0.007 \pm 0.021	0.727
Fitness Index														
S	RegOU	M	4	30.5	-51.1	30.9	0.306	1.00	-0.026 \pm 0.088	0.775				
S	RegPagel	M	4	30.2	-50.6	24.4	0.878	1.27	-0.015 \pm 0.083	0.854				
S+F	RegOU	M	5	30.6	-48.2	7.5	0.329	4.17	-0.014 \pm 0.090	0.876		0.019 \pm 0.037	0.614	
S+F	RegPagel	M	5	30.6	-48.1	7.1	0.878	4.35	-0.014 \pm 0.084	0.870		0.023 \pm 0.030	0.460	
S+P	RegOU	M	5	30.5	-48.0	6.6	0.327	4.76	-0.016 \pm 0.097	0.872	-0.007 \pm 0.043	0.870		
S+P	RegPagel	M	5	30.3	-47.5	5.2	0.874	5.88	-0.021 \pm 0.094	0.828	0.004 \pm 0.042	0.922		
S	OLS	-	3	27.3	-47.4	5.0	-	6.25	-0.156 \pm 0.095	0.115				
S	OLS	-	3	34.2	-61.4	24.1	-	1.00	-0.070 \pm 0.073	0.343				
S	RegOU	M	4	35.3	-60.6	16.3	0.052	1.47	-0.039 \pm 0.080	0.631				
S	RegPagel	M	4	35.0	-60.2	12.8	0.768	1.89	-0.021 \pm 0.072	0.774				
S+F	OLS	-	4	34.6	-59.4	8.8	-	2.70	-0.086 \pm 0.075	0.268		0.023 \pm 0.028	0.407	
S+P	OLS	-	4	34.4	-59.0	7.1	-	3.45	-0.080 \pm 0.075	0.302	0.019 \pm 0.033	0.563		
S	RegOU	C	4	34.4	-59.0	7.1	0.190	3.45	-0.068 \pm 0.079	0.397				
S+F	RegPagel	M	5	35.4	-57.8	4.0	0.770	5.88	-0.026 \pm 0.072	0.725		0.022 \pm 0.026	0.411	
S+F	RegOU	M	5	35.4	-57.8	3.9	0.063	6.25	-0.037 \pm 0.081	0.653		0.015 \pm 0.031	0.630	
S+P	RegOU	M	5	35.3	-57.5	3.5	0.059	6.67	-0.038 \pm 0.086	0.660	0.001 \pm 0.035	0.984		
S	OLS	-	3	50.8	-94.5	13.7	-	1.00	0.086 \pm 0.038	0.036				
S	RegPagel	C	4	52.1	-94.3	12.5	0.923	1.10	0.022 \pm 0.040	0.591				
S	RegOU	M	4	51.9	-93.9	9.8	0.002	1.39	0.059 \pm 0.042	0.182				
S	RegOU	C	4	51.9	-93.8	9.8	1.013	1.39	0.014 \pm 0.040	0.725				
S+P	OLS	-	4	51.7	-93.6	8.5	-	1.61	0.096 \pm 0.039	0.021	-0.022 \pm 0.017	0.207		
S+P	RegPagel	C	5	52.9	-92.9	6.0	0.929	2.27	0.038 \pm 0.042	0.382	-0.019 \pm 0.016	0.241		

Model	Character Evolution	Tree	k	lnML	AICc	w	λ or d	ER	Coefficients \pm SE			
									log SL	Predation	Flow	
S+F	OLS	-	4	51.3	-92.7	5.5	-	2.50	0.094 \pm 0.040	0.027	-0.014 \pm 0.014	0.360
S	RegPagel	M	4	51.3	-92.7	5.4	0.646	2.56	0.036 \pm 0.039	0.362		
S+P	RegOU	C	5	52.7	-92.4	4.7	1.040	2.94	0.031 \pm 0.042	0.465	-0.020 \pm 0.016	0.239
S+P	RegOU	M	5	52.4	-91.8	3.5	0.001	3.85	0.070 \pm 0.043	0.123	-0.016 \pm 0.017	0.355
S+F	RegPagel	C	5	52.3	-91.7	3.3	0.882	4.17	0.029 \pm 0.041	0.487	-0.008 \pm 0.013	0.561
S+P+F	OLS	-	5	52.3	-91.5	3.1	-	4.35	0.105 \pm 0.040	0.016	-0.022 \pm 0.017	0.206
S+P	RegPagel	M	5	52.1	-91.1	2.5	0.628	5.56	0.053 \pm 0.041	0.207	-0.021 \pm 0.018	0.260
S+F	RegOU	C	5	51.9	-90.9	2.2	0.985	6.25	0.016 \pm 0.041	0.700	-0.004 \pm 0.013	0.736
S+F	RegOU	M	5	51.9	-90.9	2.2	0.001	6.25	0.061 \pm 0.043	0.181	-0.004 \pm 0.016	0.794
Surface Area												
S	RegOU	M	4	30.2	-50.5	29.3	0.487	1.00	1.941 \pm 0.082	<0.001		
S	RegPagel	M	4	29.8	-49.7	19.6	0.927	1.49	1.946 \pm 0.081	<0.001		
S+F	RegOU	M	5	31.2	-49.3	16.4	0.613	1.79	1.893 \pm 0.078	<0.001	-0.051 \pm 0.033	0.144
S+A _{early}	RegPagel	M	5	30.8	-48.5	11.2	0.945	2.63	1.920 \pm 0.078	<0.001	-0.042 \pm 0.030	0.179
S+P	RegOU	M	5	30.7	-48.3	9.9	0.644	2.94	1.873 \pm 0.085	<0.001	0.052 \pm 0.041	0.219
S+P	RegPagel	M	5	29.9	-46.9	4.9	0.949	5.88	1.906 \pm 0.089	<0.001	0.029 \pm 0.041	0.489
S	RegOU	M	4	29.5	-49.1	23.0	0.128	1.00	1.983 \pm 0.098	<0.001		
S	RegPagel	M	4	29.0	-48.1	13.8	0.813	1.67	1.968 \pm 0.090	<0.001		
S	OLS	-	3	27.2	-47.4	9.7	-	2.38	2.040 \pm 0.095	<0.001		
S+F	RegOU	M	5	30.1	-47.3	9.2	0.218	2.50	1.959 \pm 0.095	<0.001	-0.044 \pm 0.038	0.255
S+A _{late}	RegPagel	M	5	29.9	-46.8	7.3	0.833	3.13	1.969 \pm 0.088	<0.001	-0.042 \pm 0.032	0.204
S+F	OLS	-	4	28.3	-46.6	6.5	-	3.57	2.071 \pm 0.096	<0.001	-0.048 \pm 0.035	0.187
S+P	RegOU	M	5	29.5	-46.0	4.9	0.188	4.76	1.962 \pm 0.105	<0.001	0.016 \pm 0.045	0.732
S+P	OLS	-	4	27.9	-45.9	4.7	-	5.00	2.062 \pm 0.097	<0.001	-0.046 \pm 0.042	0.280
S	RegOU	C	4	27.9	-45.9	4.6	0.325	5.00	2.024 \pm 0.104	<0.001		
S	OLS	-	3	57.0	-106.9	26.9	-	1.00	-0.068 \pm 0.030	0.035		
S	RegOU	C	4	57.5	-105.2	11.5	0.338	2.33	-0.035 \pm 0.033	0.308		
S+F	OLS	-	4	57.5	-105.2	11.3	-	2.38	-0.075 \pm 0.031	0.025	0.011 \pm 0.011	0.333

Model	Character Evolution	Tree	k	lnML	AICc	w	λ or d	ER	Coefficients \pm SE				
									log SL	Predation	Flow		
CP _{preg.}	S	RegOU	M	4	57.4	-105.0	10.3	0.000	2.63	-0.053 \pm 0.033	0.121		
	S	RegPageI	C	4	57.2	-104.4	7.8	0.784	3.45	-0.019 \pm 0.033	0.583		
	S+P	OLS	-	4	57.1	-104.2	7.1	-	3.85	-0.070 \pm 0.032	0.037	0.005 \pm 0.014	0.711
Caudal Peduncle Area													
CP _{early}	S	RegOU	M	4	37.4	-64.9	40.6	0.189	1.00	1.957 \pm 0.071	<0.001		
	S+F	RegOU	M	5	37.9	-62.7	13.7	0.122	2.94	1.955 \pm 0.073	<0.001	-0.026 \pm 0.028	0.359
	S+P	RegOU	M	5	37.4	-61.8	8.8	0.183	4.55	1.953 \pm 0.077	<0.001	0.006 \pm 0.033	0.865
	S	RegPageI	M	4	35.8	-61.8	8.6	0.861	4.76	1.922 \pm 0.068	<0.001		
	S+F	RegPageI	M	5	37.1	-61.2	6.5	0.814	6.25	1.926 \pm 0.067	<0.001	-0.037 \pm 0.024	0.142
CP _{late}	S	RegOU	M	4	36.2	-62.5	23.9	0.070	1.00	2.018 \pm 0.077	<0.001		
	S	OLS	-	3	34.4	-61.8	16.2	-	1.47	2.006 \pm 0.072	<0.001		
	S+F	OLS	-	4	35.5	-61.1	11.8	-	2.04	2.030 \pm 0.073	<0.001	-0.038 \pm 0.027	0.172
	S+F	RegOU	M	5	36.5	-60.0	6.8	0.050	3.57	2.020 \pm 0.078	<0.001	-0.021 \pm 0.029	0.482
	S	RegPageI	M	4	35.0	-60.0	6.8	0.740	3.57	1.981 \pm 0.072	<0.001		
	S	RegOU	C	4	34.8	-59.7	5.7	0.228	4.17	2.019 \pm 0.078	<0.001		
	S+P	RegOU	M	5	36.3	-59.6	5.6	0.077	4.35	2.028 \pm 0.082	<0.001	-0.014 \pm 0.034	0.686
	S+F	RegPageI	M	5	36.2	-59.3	4.9	0.705	4.76	1.992 \pm 0.073	<0.001	-0.028 \pm 0.026	0.294
	S+P	OLS	-	4	34.5	-59.2	4.5	-	5.26	2.013 \pm 0.075	<0.001	-0.015 \pm 0.032	0.647
	S	OLS	-	3	40.4	-73.7	28.2	-	1.00	0.034 \pm 0.057	0.559		
CP _{preg.}	S+P	OLS	-	4	41.1	-72.3	13.9	-	2.04	0.048 \pm 0.058	0.421	-0.028 \pm 0.025	0.272
	S	RegOU	M	4	40.6	-71.3	8.3	0.001	3.45	0.059 \pm 0.065	0.374		
	S+F	OLS	-	4	40.6	-71.3	8.3	-	3.45	0.026 \pm 0.060	0.671	0.012 \pm 0.022	0.575
	S	RegOU	C	4	40.5	-71.1	7.7	0.164	3.70	0.054 \pm 0.062	0.391		
	S	RegPageI	M	4	40.4	-71.0	7.1	0.606	4.00	0.057 \pm 0.059	0.348		
S+P	RegOU	M	5	41.4	-69.8	3.9	0.001	7.14	0.077 \pm 0.066	0.261	-0.031 \pm 0.026	0.258	

Chapter 2

Interspecific life history variation in livebearing female fishes of the genus *Gambusia* (Poeciliidae)

Abstract: Evolutionary processes may form a continuum from micro- to macroevolutionary scales, such that microevolutionary processes can explain macroevolutionary patterns. Here we analyze macroevolutionary pattern of life history variation in female *Gambusia*. Life history theory, largely developed and tested at the microevolutionary level, predicts that an increase in mortality later in life reduces age at maturity and increases reproductive allocation before and during the age when mortality increases. Life history theory also predicts that higher mortality at later ages selects for more and smaller embryos. Predation and flow are key factors for freshwater fish, in part because of the high mortalities that they can exert. We used life history theory to propose how life history in *Gambusia* could respond to variation in predation and flow regimes. We collected life history data from preserved specimens of 20 *Gambusia* species and found remarkable life history variation across the genus. At the intraspecific level, life history traits were related to female body size and stage of embryo development. However, these allometric and pregnancy correlations varied among species. At the interspecific level, the best predictor of life history variation was female body size. Moreover, body size also explained the allometric variation in life-history traits among collections. The effect of predation on life histories coincided with

most of the predictions from life-history theory: species from high-predation sites tended to have larger gonads and more and smaller embryos. However, the differences of species between high-and low-predation species were relatively small. Latitude, flow, predation intensity, and female body size showed statistically significant phylogenetic signal, but life history traits did not.

Introduction

A general concept in evolutionary biology is that adaptation at the microevolutionary level (within species) can explain macroevolutionary patterns of variation (among higher taxa; Darwin 1872: 49; Watson et al. 1936: 58; Charlesworth et al. 1982; Arnold et al. 2001). Under this view, the same selective factors can drive populations and higher taxa in similar directions towards optimal areas of the adaptive space, forming a continuum between micro- and macroevolution (Charlesworth et al. 1982). However, it has been suggested that the direction of adaptive divergence can be modified by other evolutionary processes (e.g. random genetic drift), which can blur microevolutionary patterns at the long term (Stanley 1979: 189). Moreover, some evolutionary scenarios may not fit the concept of a micro-macroevolutionary continuum. For example, the direction of selection across the adaptive landscape may not correspond between micro-and macroevolutionary scales if the adaptive landscape peaks vary in position among higher taxa (Arnold et al. 2001) or if different taxa find alternative morphological solutions to the same functional problem (Losos 2011). These points favor an alternative view that microevolutionary divergence cannot always be extrapolated to explain divergence at the macroevolutionary scale (Goldschmidt 1940; Van Valen 1974; Eldredge and Cracraft 1980). A useful approach to test the connection between micro- and macroevolutionary scales is to produce models of divergence from microevolutionary patterns to predict

patterns at the macroevolutionary level (Arnold et al. 2001; Losos and Ricklefs 2009). We used this strategy to explain the interspecific patterns of life history in female *Gambusia*, testing predictions derived from models of life history evolution that were developed to assess microevolutionary processes.

Explaining the remarkable diversity of life histories in nature has been addressed with a large body of theory based on models at the population level (Roff 1992; Stearns 1992; Roff 2002). Theoretically, natural selection should act upon life-history variation to maximize Darwinian fitness (Cole 1954). Organisms are expected to invest optimal proportions of their limited resources into different body compartments (growth, reproduction, maintenance; Williams 1966). Thus, an increase in mortality at a given age is predicted to reduce age at maturity (Gadgil and Bossert 1970), and increase reproductive allocation before and during the age when mortality increases (Gadgil and Bossert 1970; Schaffer 1974; Law 1979; Michod 1979). Life history theory also predicts that higher mortality at later ages selects for smaller embryos (Sibly and Calow 1986). Given a necessary trade-off between number and size of offspring (Roff 2002; p. 257), higher mortality late in life is expected to simultaneously increase the number of embryos as embryo size decreases.

These predictions are largely supported by intraspecific field studies of various species found across gradients of predation in fishes (*Poecilia reticulata*: Reznick et al. 1996 and references herein; *Gambusia hubbsi*: Downhower et al. 2000; *Brachyrhaphis rhabdophora*: Johnson and Belk 2001; *Brachyrhaphis*

episcopi: Jennions and Telford 2002; Rivulus hartii: Walsh and Reznick 2009), an amphipod (*Hyalella azteca*: Wellborn 1994), a copepod (*Daphnia ambigua*: Walsh and Post 2011), and a lizard (*Sceloporus undulatus*: Tinkle and Ballinger 1972). These predictions are also supported by laboratory (*Drosophila melanogaster*: Gasser et al. 2000) and field (*P. reticulata*: Reznick et al. 1990) experimental evolution studies that have manipulated mortality rate and measured evolved changes in life history phenotype. Moreover, data from fisheries are consistent with these models. Maturation at younger age is observed as a by-product of heavy fishing pressure - where larger fish are often the most intensively harvested (Sharpe and Hendry 2009). Here we extend life history predictions to the interspecific level, asking if life histories of species living in contrasting environmental regimes vary as predicted by theory. At the same time, we incorporate other, potentially interacting, factors that might affect life history evolution.

Predation, latitude, and disturbance regimes are potentially important environmental factors in shaping life histories. Predation is a key factor in nature (Lima 1998; Langerhans 2006; Hawlena and Schmitz 2010), structuring communities (Paine 1966; Sih et al. 1985), acting as a selective factor that induces phenotypic divergence (Endler 1995; Vamosi 2005), and influencing life history patterns in fishes (see above). Latitude is also associated with life history patterns in fishes (Vila-Gispert et al. 2002; Heibo et al. 2005), squamates (James and Shine 1988; Angilletta et al. 2004), and birds (Griebeler et al. 2010). This

relationship may be a by-product of higher predation levels at lower latitudes (Sih et al. 1985; Pennings and Silliman 2005), or a consequence of resource seasonality or length of breeding seasons (Griebeler et al. 2010). Flow regime is one of the most important disturbance factors in freshwaters (Poff et al. 1997). The correlation between flow regime and life history variation has been proposed (Johnson and Bagley 2011), but has yet to be tested. Floods in mountain rivers cause high mortalities, specially in fish of smaller size (Chapman and Kramer 1991; Grether et al. 2001). Theoretically, these high differential mortalities could have an effect on life history evolution.

The genus *Gambusia* is an excellent system for a macroevolutionary life-history study. *Gambusia* species are naturally distributed along a large latitudinal gradient from North America to Northern Colombia (Rosen and Bailey 1963; Lucinda 2003). Moreover, many species are restricted to distinct predation and flow regimes (Langerhans et al. 2007). For example, *G. alvarezi* lives only in ponds with other non-piscivorous fish, whereas *G. luma* lives in streams with multiple predatory fish in the community. These gradients have promoted phenotypic divergence in *Gambusia*, where predation (Langerhans et al. 2007; Chapter 1), and flow regime (Langerhans and Reznick 2010; Chapter 1) are related to body shape. Here we study the effects of predation and flow regime on life history variation of *Gambusia*, using the same specimens previously used in analyses of body shape variation (Chapter 1).

Previous studies on several *Gambusia* species demonstrated a large range of life history variation in the genus (e.g., Hubbs and Mosier 1985; Reznick and Miles 1989; Downhower et al. 2000; Riesch et al. 2010a; Riesch et al. 2010b; Johnson and Bagley 2011; Pires et al. 2011a). However, few of these data were useful to test our adaptive hypothesis for three reasons. First, our main objective was to study interspecific variation across the genus, while most previous reports focused on intraspecific variation. Second, previous studies have methodological differences that make comparison difficult (e.g., studies differ in the way they account for the effect of female body size). Third, one of our aims was to use a multivariate approach to account for covariation among life history traits, while accounting for size and pregnancy. Previous studies vary in the traits analyzed and they dealt with the effect of size and pregnancy in different ways.

Our main objective was to analyze interspecific life history variation in female *Gambusia* using 20-species from varying predation and flow regimes. Our survey includes those *Gambusia* species that show little intraspecific habitat variation. We analyzed size at maturity, embryo size, fecundity, reproductive allotment, and mode of maternal provisioning (matrotrophy). Additionally, the variation in intraspecific allometric coefficients of embryo size, fecundity, and ovary weight were examined. We analyzed life history traits individually and, to account for their correlation, as composite variables. We used phylogenetically

informed statistical methods to test whether interspecific life history variation is explained by female body size, predation, flow, and latitude.

Methods

Source of specimens and habitat characterization

We analyzed preserved specimens from museum and personal collections (Table 2.1). The same collections were used for a study of body shape (Chapter 1), where further details about sampling, localities, and habitat characterization can be obtained (Table 1.1 and Appendix 1.1). Briefly, predation and flow regimes of each locality were determined by analyzing original field notes. The predation regime of each site was estimated from the inventory of the fish community at that site. Low-predation (LP) localities had only non-piscivorous fish and high-predation (HP) sites had piscivorous fish. Flow regime was characterized into two levels, low flow (LF, lakes and ponds) and high flow (HF, streams). Geographic coordinates were obtained from plots of data in Google Earth (Google Inc., 2011). Latitude of merged collections (see below) was the average of the original collections' latitudes. After merging collections from the same species that had nearby sites (Table 2.1) there were a total of 1012 specimens representing 20 species from 26 locations. The number of females was reduced to 695 after calculating the minimum size at maturity and excluding females that had partial broods (details below) or were not pregnant.

Life history data

Following Pires et al. (2011b), we measured female standard length ($SL \pm 0.1$ mm), female wet weight in alcohol ($W_{\text{fem}} \pm 0.001$ g), fecundity (N_{emb} , number of embryos), number of broods, ovarian tissue dry weight ($W_{\text{ovary}} \pm 0.01$ mg), mean embryo weight (W_{emb} , dry weight of batch of embryos/fecundity, to the nearest 0.01 mg), and stage of development of embryos. Embryo development was measured using the scale of Haynes (1995) as modified by Pires et al. (2011b). This scale goes from egg (fertilized or not, Stage 0) to embryo ready for birth (Stage 50). When females had an advanced batch of embryos and a group of unfertilized vitellogenic eggs, the weight of the unfertilized eggs were added to the ovarian tissue weight and only the advanced group of embryos was counted as a brood (Turner 1940).

Statistical analysis: estimation of variables

Minimum size at maturity (SL_{min}) was estimated for each collection as the SL of the smallest pregnant female with developing embryos (Stage ≥ 2). After calculating SL_{min} , non-pregnant females were excluded from all analyses. Females with eggs only (Stage 0) were considered non-pregnant because we could not unambiguously discriminate between fertilized and unfertilized eggs. Four females with partially recruited broods and nine with partially spawned broods were excluded because their actual fecundities are unknown. They were

recognized, respectively, by their broods at early- (Stage≤10) or late-stage (Stage 45-50) and by their low fecundities (relative to other females of the same size).

The effects of intraspecific allometry and pregnancy condition on individual-based life history traits (N_{emb} , W_{emb} , and W_{ovary}) were accounted for by estimating trait values for the hypothetical average-size female late in pregnancy (Stage 50) for each collection. The estimated values were obtained from linear models (Monteiro 1999; Berner 2011), as follows:

$$\log \textit{trait} = I + \varepsilon$$

$$\log \textit{trait} = I + \beta_{\text{size}}(\log \text{SL}) + \varepsilon$$

$$\log \textit{trait} = I + \beta_{\text{stage}}(\text{stage}) + \varepsilon$$

$$\log \textit{trait} = I + \beta_{\text{size}}(\log \text{SL}) + \beta_{\text{stage}}(\text{stage}) + \varepsilon,$$

where I is the intercept, *trait* is each life history trait, β is the respective coefficient, stage is the stage of embryo development, and ε is the error term. Fitting of models to data was quantified with small sample corrected Akaike information criterion (AICC; Anderson and Burnham 2002). The amount of evidence explained by each model was estimated with Akaike weights (Anderson 2008), Bayesian posterior probabilities (i.e. model probabilities) that range from zero (model not explaining variation) to one (model fully explaining variation). Average coefficients and their respective standard errors were calculated as the respective values obtained from each model, weighted with their respective Akaike weight. Model averaging was performed with unconditional estimation (using all models instead of only those including each parameter; Anderson

2008; Grueber et al. 2011). The estimated life history values (Table 2.1) and their respective allometric coefficients (β_{size}) were used in further analyses. The allometric coefficients (β_{size}) of N_{emb} and W_{ovary} were considered isometric when if they were equal to three because fecundity and ovary size are a function of body volume, which scales to the third power of SL (Calder 1984). Hence, allometry was positive when $\beta_{\text{size}} > 3$ and negative when $\beta_{\text{size}} < 3$.

Viviparous organisms, such as *Gambusia*, can allocate nutrients to embryos throughout the course of pregnancy (Wourms et al. 1988). Modes of allocation vary from lecithotrophy, where mothers allocate nutrients to embryos prior to fertilization via fully yolked eggs, to matrotrophy, where mothers continually provide nutrients to embryos during gestation (Wourms 1981). Matrotrophy ranges from unspecialized, when embryos maintain their weight during gestation using maternal provisioning that compensates metabolic mass loss, to extreme, where embryos increase in weight several times during development (Wourms 1981). Extreme matrotrophy has evolved more than 30 times in vertebrates (Dulvy and Reynolds 1997), including in Poeciliidae (Reznick et al. 2002; Pollux et al. 2009; Pires et al. 2010; Pires et al. 2011b). Some *Gambusia* species appear to show unspecialized matrotrophy (Marsh-Matthews et al. 2005), although a more thorough survey of matrotrophy of the genus has not been accomplished. In this study we quantify the degree of maternal provisioning in many species of *Gambusia* allowing for a fuller understanding of their reproductive mode.

We quantified maternal provisioning using the Matrotrophy Index (MI), a measurement of the change in dry embryo weight between fertilization and parturition (Scrimshaw 1945; Wourms et al. 1988; Blackburn 1994; Reznick et al. 2002). It is calculated as W_{emb} estimated at birth (Stage 50) divided by W_{emb} estimated at fertilization (Stage 0). These estimated values were obtained from a linear regression between W_{emb} and stage of embryo development. We evaluate whether MI of each collection was higher than 0.7 following Reznick et al. (2002). This threshold of 0.7 is derived from the observation that eggs of oviparous fish species lose on average 30% of their dry weight during gestation due to metabolism (Wourms 1981). This threshold value is not critical- a value of 0.6 is not much different from 0.8. However, it is a reasonable benchmark for comparative purposes.

To determine whether life histories match a fast-slow continuum pattern, life history variation was condensed using a Principal Component Analysis (PCA) based on the estimated life-history values for each collection. If life histories form a fast-slow continuum a single principal component (PC) will account for most of the life history variation (Bielby et al. 2007), once interspecific allometry has been accounted for. The variables included in the PCA were SL, SL_{min} , N_{emb} , W_{emb} , W_{ovary} , all log-transformed. Principal components with eigenvalues larger than one were retained (Tabachnick and Fidell 2001). Scores of retained PCs were used in further analyses. PCA results were represented with Burnaby Back

Projection (Berner 2011), which allows to project variation of each PC in an orthogonal plane from previous PCs.

Phylogenetic analysis

For each morphological trait (log SL, log W_{fem} , log SL_{min} , log N_{emb} , log W_{emb} , log W_{ovary} , MI, $\beta_{\text{size-Nemb}}$, $\beta_{\text{size-Wemb}}$, $\beta_{\text{size-Wovary}}$, MI, and PC scores), we evaluated five adaptive models (models that include independent variables putatively indicating variation in the selective regime):

$$\text{trait} = I + \log \text{SL} + \varepsilon$$

$$\text{trait} = I + \log \text{SL} + \textit{predation} + \varepsilon$$

$$\text{trait} = I + \log \text{SL} + \textit{flow} + \varepsilon$$

$$\text{trait} = I + \log \text{SL} + \textit{latitude} + \varepsilon$$

$$\text{trait} = I + \log \text{SL} + \textit{predation} + \textit{flow} + \textit{predation*flow} + \varepsilon.$$

The covariate log SL was dropped from the models to avoid autocorrelation when the trait was log SL, log female wet weight, or PC scores.

We tested three regression models of character evolution (i.e. error structure). The first model was Ordinary Least Squares (OLS), equivalent to a non-phylogenetic approach (i.e., assuming a star phylogeny with no hierarchical structure and contemporaneous tips). The second model (RegPagel; Pagel 1999; Garland et al. 2005; Lavin et al. 2008) begins with a specified hierarchical phylogenetic tree and simultaneously estimates parameter coefficients and λ , a factor that multiplies the off-diagonal elements of the variance-covariance matrix

of relatedness (Freckleton et al. 2002). The value λ indicates strength of phylogenetic signal, from zero (no signal) to one (phylogeny fully explains error structure). The third model was the Ornstein–Uhlenbeck process (RegOU; Lavin et al. 2008), which assumes that divergence tends to be restrained back towards an optimum determined by selection (Garland et al. 1993; Hansen 1997; Butler and King 2004). In this model the regression coefficients and a measure of phylogenetic signal (d) are again estimated simultaneously (Lavin et al. 2008). The value of d ranges from zero (no signal) to one (phylogeny fully explains error structure), to more than one (the optimal error structure comes from a tree that is more hierarchical than the original).

We also used a univariate measure of phylogenetic signal, the parameter K of Blomberg et al. (2003). Values of K range from zero (variation independent of phylogeny), to less than one (variation departing from Brownian motion), to one (resemblance among relatives fits the expectation of Brownian motion evolution, averaged across the phylogeny), to more than one (resemblance among relatives is higher than expected under Brownian motion). For each variable analyzed, we calculated K and its probability of being larger than zero. This was performed with a randomization procedure that calculates the probability of $K > 0$ as the frequency that the observed phylogenetically correct mean (MSE) was larger than n randomized MSE (Blomberg et al. 2003). In this study, $n = 9,999$ replicates. These analyses were performed using *phylosignal* in the R package *picante* (Kembel et al. 2010).

The *Gambusia* phylogeny was the same as that of Chapter 1 (B. Langerhans, unpub., Appendices 1.5-1.7). This molecular phylogeny is a majority (50%) consensus based upon mitochondrial (ND2, Cyt b) and nuclear (S7) genes. All phylogenetic tests were performed twice, the first time with the original branch lengths (proportional to the units of inferred nucleotide substitution) and the second time with branch lengths set to a constant value of one. The latter alternative reduces the magnification of phenotypic differences that occur when contrasting closely related taxa and assumes that variation occurred when clades branch (punctuated evolution). The *Gambusia* phylogeny had three soft polytomies (multifurcations resulting from lack of resolution at a given node), which may increase the Type I error when calculating the significance of F-values for each coefficient of GLS models. We corrected for polytomies by reducing the degrees of freedom with the equation $N-k-1-z$, where N are the number of tips in the phylogeny, k the number of parameters estimated for the respective model, and z (five in this study) are the number of branches that were set to zero length (i.e., polytomies; Garland and Díaz-Uriarte 1999).

The most supported regression models were chosen using model selection, preferring the models within $\Delta AIC_C=4$. This set of best-supported models was averaged with unconditional estimation (including all models from top set) by weighting coefficients with the respective Akaike weight (Anderson 2008). To facilitate the comparison between models, Evidence Ratios (ER; Anderson 2008) were calculated as $w_{\text{best model}}/w_i$, indicating how many times

better a model is against the i -th model. OLS, RegPagel, and RegOU analyses were performed using the Matlab program RegressionV2.m (A. R. Ives and T. Garland Jr.; Lavin et al. 2008). Other analyses used R (R Development Core Team 2011), implementing the package *ape* (Paradis et al. 2004), and code from Berner (2011).

Results

Life history variation

Female *Gambusia* showed a large range of interspecific life history variation. The ranges of individual variation were 17.84-53.30 mm for SL (a 2.99-fold), 0.033-3.127 g for W_{fem} (a 94.758-fold), 1-47 for N_{emb} , 0.46-8.12 mg for W_{emb} (a 17.66-fold), and 1.90-195.53 mg for W_{ovary} (a 102.91-fold). Table 2.1 shows the interspecific ranges of variation after accounting for intraspecific allometry and pregnancy condition. Maternal provisioning was lecithotrophic for most species, but incipient matrotrophy was observed in *G. atrora*, *G. luma*, *G. rhizophorae*, and *G. vittata* (Table 2.1). Only one female exhibited more than one brood (*G. nicaraguensis*, UMMZ 199689, with 16 embryos at stage 20 and eight at stage 45). This female was excluded from further analyses.

At the intraspecific level, body size and pregnancy condition accounted for a considerable proportion of variation in N_{emb} , W_{emb} , and W_{ovary} (Appendix 2.1). In most species, larger females had more embryos and heavier ovaries (positive

$\beta_{\text{size}} N_{\text{emb}}$ and $\beta_{\text{size}} W_{\text{ovary}}$ values, respectively). However, the proportionality between female size and fecundity and gonadal weight showed considerable variation. The allometric coefficient of N_{emb} ($\beta_{\text{size}} N_{\text{emb}}$) varied from negative allometry ($\beta_{\text{size}} N_{\text{emb}} < 3$ in three collections of three species) to isometry ($\beta_{\text{size}} N_{\text{emb}} = 3$) in remaining collections. $\beta_{\text{size}} W_{\text{ovary}}$ varied from negative allometry ($\beta_{\text{size}} W_{\text{ovary}} < 3$ in *G. aurata*), to isometry ($\beta_{\text{size}} W_{\text{ovary}} = 3$), to positive allometry ($\beta_{\text{size}} W_{\text{ovary}} > 3$ in nine collections). W_{emb} increased with female body size ($\beta_{\text{size}} W_{\text{emb}} > 0$) in eight collections of six species. The number of embryos remained constant through pregnancy ($\beta_{\text{stage}} N_{\text{emb}} = 0$) except for three collections with embryo loss ($\beta_{\text{stage}} N_{\text{emb}} < 0$). In the majority of collections, embryo and gonadal weight decreased throughout pregnancy ($\beta_{\text{stage}} W_{\text{emb}}$ and $\beta_{\text{stage}} W_{\text{ovary}} < 0$), but in others these weights remained constant.

Interspecific analyses showed patterns of correlation among traits and a phylogenetic effect on environmental factors and body size but not on life-history traits. PCA produced two PCs that condensed 94% of variation (Table 2.2). PC 1 was strongly related to body size; species with larger mean female size matured at larger sizes and had heavier embryos and ovaries. PC 2 showed a negative correlation between fecundity and embryo weight (Figure 2.1). Phylogenetic signal (K) was high for latitude, low for predation and size variables (SL, W_{fem} , PC 1), and negligible for life history traits (Table 2.3). Regression results showed that the best-supported models for log SL, log W_{fem} , log SL_{min} , and PC 1 were RegOU models with low levels of phylogenetic signal ($d=0.007-0.159$). The best-

supported models for the remaining life-history traits were non-phylogenetic (Table 2.4). For the RegOU and RegPagel models, the phylogenetic tree that best-fit the data was the molecular phylogeny with branch lengths proportional to nucleotide substitution rate.

At the interspecific level, body size and predation were correlated with life history traits. Larger species had heavier embryos and heavier ovaries (Table 2.4). Larger species also had higher $\beta_{\text{size}} N_{\text{emb}}$ and $\beta_{\text{size}} W_{\text{emb}}$ (Table 2.4), indicating that when they grow their fecundities and reproductive allocations increase at a higher rate. As predicted by theory, high predation species tended to have higher fecundities ($\log N_{\text{emb}}$) and heavier gonads (W_{emb}). However, these patterns were not significant. Predation was correlated with $\beta_{\text{size}} W_{\text{ovary}}$ (Table 2.4, Figure 2.3), suggesting that HP species allocate proportionally more to ovaries with growth. Flow or latitude were not strong predictors of any life history trait.

Discussion

We found remarkable life history variation across *Gambusia*, in agreement with previous reviews of poeciliid life history (Johnson and Bagley 2011; Pires et al. 2011a). At the intraspecific level, female body size and stage of embryo development proved to be significant factors for life histories of many species, although in variable ways. At the interspecific level, phylogeny had a small effect on female body size but its effect on life history traits was null. The best predictor

of interspecific life history variation was female body size and predation. Body size also explained the allometric variation in life-history traits among collections (β_{size} coefficients). The effect of predation on life histories coincided with some of the predictions (high predation species tended to have more embryos and heavier gonads), but that pattern was tenuous. Interestingly, we observed a rare life-history pattern, high-predation species allocated proportionally more to ovaries when growing (i.e., high-predation was correlated with high $\beta_{\text{size}} W_{\text{ovary}}$).

The response of interspecific life history of female *Gambusia* to predation regime showed a pattern that partially matched the predictions derived from life history theory. We predicted that HP females would have lower size at maturity, higher fecundity, smaller embryos, and higher reproductive allocation. Instead, we observed a tenuous pattern of HP females having larger size at maturity, higher fecundity, and higher reproductive allocation (Table 2.4). This pattern is blurry in comparison with the sharp contrasts in life histories that have been documented at the intraspecific level for several species living along predation gradients (e.g., Reznick and Endler 1982). A mischaracterized predation regime may be responsible of this blurry pattern. However, we observed in Chapter 1 a clear effect of predation on female body shape of these same females analyzed here. Moreover, predation was strongly correlated with one life history trait ($\beta_{\text{size}} W_{\text{ovary}}$, further discussion below). This mitigated predictive power of life history theory at the macroevolutionary level may result from the nature of life history traits. These traits experience strong levels of selection in different directions

from multiple ecological factors (Arnold et al. 2001). This strong selection erodes additive genetic variation, what may explain the common observation of low heritabilities in life history traits (Roff 1992: 12). In agreement with this idea of selection quickly moving life history phenotypes in multiple directions, life history traits typically show low levels of phylogenetic signal (Blomberg et al. 2003; this study). This implies that close phylogenetic relatedness accounts for a small proportion of the larger observed variation in these traits. If life history traits are influenced by multiple selective factors, the adaptive landscape of life history traits is relatively flat, as it has been suggested (Arnold et al. 2001). In this case, the direction of evolutionary divergence may differ between micro- and macroevolutionary scales, what would explain the mismatch between this study and the previous ones about the effect of predation on life histories. Only the negative correlation between fecundity and embryo size showed a continuum between evolutionary scales (Table 2.2, Figure 2.1). This negative association between fecundity and embryo size has been previously observed in populations of some poeciliid species (e.g., Riesch et al. 2010b; Ponce de León et al. 2011), and there is accumulating evidence on the trade-off between number and size of offspring in multiple organisms at different taxonomic scales (Smith and Fretwell 1974; Roff 2002; p. 257).

Flow was not related to *Gambusia* life history. Two issues could obscure a possible correlation between flow and life history. First, there is a negative correlation between predation and flow regimes in the samples that we studied

here ($r=-0.75$, Chapter 1). Such high correlation indicate multicollinearity (Slinker and Glantz 1985). Therefore, linear models that had both factors as independent variables could have results with one factor obscuring the effect of the other (Tabachnick and Fidell 2001). However, flow was not a good predictor of life histories, even when it was the only dependent variable in the model (Table 2.2). The second issue was the flow regime was classified coarsely, contrasting lotic (streams) vs. lentic (lacustrine) habitats. This typically used contrast is problematic because lotic and lentic systems differ in multiple aspects beside flow velocity (Wetzel and Likens 2000). Moreover, each habitat may vary spatially (microhabitat heterogeneity; Church 2002; Wohl and Merritt 2008) or temporarily (flow seasonality; Sabo and Post 2008). Further studies on the effect of flow on the phenotype of *Gambusia* could refine this characterization of flow with more hydrologically-informed methods (e.g., Sabo and Post 2008).

Latitudinal values were related with *Gambusia's* phylogeny but not with its life history. Latitudinal distribution is commonly related with phylogenetic relatedness (Blomberg et al. 2003; Rezende et al. 2004; Oufiero et al. 2011), indicating that closely related species are distributed in proximity to their closer relatives. Latitude is also commonly related to life history patterns in vertebrates (James and Shine 1988; Vila-Gispert et al. 2002; Angilletta et al. 2004; Heibo et al. 2005; Traynor and Mayhew 2005; Griebeler et al. 2010), although the relation sometimes is absent (Hubble 2003). The underlying explanation for this pattern is not clear. One alternative is that predation is higher at lower latitudes (Pennings

and Silliman 2005). Here, regardless of the wide latitudinal range covered by our sampling ($> 18^\circ$ of latitude), we found no correlation of life histories with latitude.

Body size was a strong predictor of some aspects of *Gambusia* life histories (Appendix 2.1, Table 2.4). Larger female size was correlated with higher fecundities among females within each collection and among species (the latter marginally). Similarly, female body size was related with ovary weight at intra- and interspecific levels. The correlations between body size and fecundity, and body size and reproductive investment are very common patterns in nature (Roff 1992; Sokolovska et al. 2000; Roff 2002). These correlations may indicate that larger females can invest more in reproduction because they have more abdominal space, or are better able to acquire resources (Roff 1992). Regarding embryo size, species with larger females had larger embryos. The correlation between female body size and propagule size is common in nature, although its functional explanation is not fully understood (Roff 1992: 347; 2002 :171; Davis et al. 2012). Larger offspring may have higher fitness, e.g., due to higher survivorship (Roff 2002: p. 258), and larger females may have more space or resources to allocate to each embryo (Forsman and Shine 1995). We did not find support for the idea that female body size limits embryo size in *Gambusia*. If abdominal size was the limitation for embryo size, then species with larger females would have shown the least increase in embryo size with increases with body size ($\beta_{\text{size}} W_{\text{emb}}$ smaller in larger species), but we found a marginally significant opposite trend (Table 2.4).

Allometric coefficients of life history traits exhibited a relation with body size and, in the case of ovarian weight, with predation. The adaptive significance of allometric coefficients have been frequently studied in the context of sexual selection. Typically, larger individuals exhibit larger sexually-selected traits (Baker and Wilkinson 2001). In contrast, in ecomorphological analysis, variation in allometric coefficients is commonly seen as an inconvenience in the data rather than data itself (e.g., heterogeneity of slopes is a violation of an ANCOVA assumption). Here we took a different approach, accounting for allometric heterogeneity and testing the effect of body size and environmental factors on the allometric coefficients. We found that females of larger species increase in fecundity, embryo size, and ovary size at greater rates as they grow (Table 2.4). The latter also occurs in *Gambusia affinis*, where females invest more in reproduction at larger body sizes (Billman 2011). Few studies have tested the effect of an ecological factor on allometry (Kelly et al. 2000; Jennions and Kelly 2002; Egset et al. 2011), and this is the first to do so with life history traits or at the interspecific level. Previous studies have analyzed the allometry of morphological characters, across populations, and in relation with predation regime. They found no differences among populations (Egset et al. 2011), or small differences not related with predation (Kelly et al. 2000; Jennions and Kelly 2002). Here we found considerable allometric differences among species (Appendix 2.2), and an effect of predation on the allometric coefficient of ovary

weight (Table 2.4, Figure 3). To the best of our knowledge, this is the first report of an allometric coefficient responding to an environmental selective regime.

The pattern of covariation among life history traits in *Gambusia* did not fit a "fast-slow continuum". Macroevolutionary patterns of life history variation have been traditionally fitted along a "pace of life" axis, from "slow" to "fast" life histories. Few studies have assessed the prevalence of this continuum, and fewer still do so while accounting for phylogeny (Clobert et al. 1998; Sæther and Bakke 2000; Bielby et al. 2007). Two life history traits in *Gambusia* aligned along a fast-slow continuum: species with higher fecundities had smaller embryos (a fast pace of life). However, fast-pace-of-life female *Gambusia* wither did not mature at smaller sizes or have larger reproductive allocation, two characteristics expected in a fast pace of life mode. The absence of these traits in the life history axis was not likely because of a lack of variation (Table 2.1). As an alternative, this could be another example of the variability in the patterns of life history trait covariation (Jeschke and Kokko 2009), which, in addition to reports of life history alignment along more than one axis of variation (Bielby et al. 2007), limit the utility of the slow-fast continuum to represent life histories.

Maternal provisioning showed little variation in *Gambusia* (MI range from 0.45 to 1.24). These values indicate that maternal provisioning within the genus ranges from lecithotrophy to incipient matrotrophy, as previously concluded for some species of *Gambusia* in studies with radiolabeled transfer methods (Marsh-Matthews et al. 2010). Another reproductive strategy found in some poeciliid

fishes is superfetation - a pregnant female simultaneously carrying two or more litters of embryos at different developmental stages. Superfetation has been observed in several families of viviparous fish including Poeciliidae and it has been reported for few species of *Gambusia* (Turner 1940; Scrimshaw 1945; but see Meffe 1985). In our survey we only found one superfetation female (of *G. nicaraguensis*). Our results indicate that, in the species of *Gambusia* analyzed, superfetation is a rare irregularity expressed in few specimens rather than a regular mode of breeding. Such rare cases of isolated individuals expressing superfetation have previously been reported for some members of Poeciliidae (Hubbs 1971; Meffe 1985; Pires et al. 2011a; DNR, pers. obs.).

Literature Cited

- Anderson, D. R. 2008. Model Based Inference in the Life Sciences: A Primer on Evidence. Springer, New York, NY.
- Anderson, D. R., and K. P. Burnham. 2002. Avoiding pitfalls when using information-theoretic methods. *The Journal of Wildlife Management* 66:912-918.
- Angilletta, M. J. J., P. H. Niewiarowski, A. E. Dunham, A. D. Leaché, and W. P. Porter. 2004. Bergmann's clines in ectotherms: illustrating a life-history perspective with Sceloporine lizards. *The American Naturalist* 164:E168-E183.
- Arnold, S. J., M. E. Pfrender, and A. G. Jones. 2001. The adaptive landscape as a conceptual bridge between micro- and macroevolution. *Genetica* 112-113:9-32.
- Baker, R. H., and G. S. Wilkinson. 2001. Phylogenetic analysis of sexual dimorphism and eye-span allometry in stalk-eyed flies (Dipsidae). *Evolution* 55:1373-1385.
- Berner, D. 2011. Size correction in biology: how reliable are approaches based on (common) principal component analysis? *Oecologia* 166:961-971.
- Bielby, J., G. M. Mace, O. R. P. Bininda-Emonds, M. Cardillo, J. L. Gittleman, K. E. Jones, C. D. L. Orme, and A. Purvis. 2007. The fast-slow continuum in

- mammalian life history: an empirical reevaluation. *The American Naturalist* 169:748-757.
- Billman, E. J. 2011. Changes in Life History within an Individual's Lifetime. Department of Biology. Brigham Young University.
- Blackburn, D. G. 1994. Standardized criteria for the recognition of embryonic nutritional patterns in squamate reptiles. *Copeia* 1994:925-935.
- Blomberg, S. P., T. Garland, Jr, and A. R. Ives. 2003. Testing for phylogenetic signal in comparative data: behavioral traits are more labile. *Evolution* 57:717-745.
- Butler, M. A., and A. A. King. 2004. Phylogenetic comparative analysis: a modeling approach for adaptive evolution. *Am. Nat.* 164:683-695.
- Calder, W. A., III. 1984. Size, Function, and Life History. Harvard University Press, Cambridge, Massachusetts.
- Chapman, L. J., and D. L. Kramer. 1991. The consequences of flooding for the dispersal and fate of poeciliid fish in an intermittent tropical stream. *Oecologia* 87:299-306.
- Charlesworth, B., R. Lande, and M. Slatkin. 1982. A Neo-Darwinian commentary on macroevolution. *Evolution* 36:474-498.
- Church, M. 2002. Geomorphic thresholds in riverine landscapes. *Freshwat. Biol.* 47:541-557.

- Clobert, J., T. Garland Jr, and R. Barbault. 1998. The evolution of demographic tactics in lizards: a test of some hypotheses concerning life history evolution. *J. Evol. Biol.* 11:329-364.
- Cole, L. C. 1954. The population consequences of life history phenomena. *The Quarterly Review of Biology* 29:103-137.
- Darwin, C. 1872. *On the origin of species by means of natural selection*. Murray, London, UK.
- Davis, R. B., J. Javoiš, J. Pienaar, E. Õunap, and T. Tammaru. 2012. Disentangling determinants of egg size in the Geometridae (Lepidoptera) using an advanced phylogenetic comparative method. *J. Evol. Biol.* 25:210-219.
- Downhower, J. F., L. P. Brown, and M. L. Matsui. 2000. Life history variation in female *Gambusia hubbsi*. *Environ. Biol. Fishes* 59:415-428.
- Dulvy, N. K., and J. D. Reynolds. 1997. Evolutionary transitions among egg-laying, live-bearing and maternal inputs in sharks and rays. *Proceedings of the Royal Society of London. Series B: Biological Sciences* 264:1309-1315.
- Egset, C. K., G. H. Bolstad, G. Rosenqvist, J. A. Endler, and C. Pélabon. 2011. Geographical variation in allometry in the guppy (*Poecilia reticulata*). *J. Evol. Biol.* 24:2631-2638.
- Eldredge, N., and J. Cracraft. 1980. *Phylogenetic Patterns and the Evolutionary Process*. Columbia University Press, New York, NY.

- Endler, J. A. 1995. Multiple-trait coevolution and environmental gradients in guppies. *Trends in Ecology & Evolution* 10:22-29.
- Forsman, A., and R. Shine. 1995. Parallel geographic variation in body shape and reproductive life history within the Australian scincid lizard *Lampropholis delicata*. *Funct. Ecol.* 9:818-828.
- Freckleton, R. P., P. H. Harvey, and M. Pagel. 2002. Phylogenetic analysis and comparative data: a test and review of evidence. *Am. Nat.* 160:712-726.
- Gadgil, M., and W. H. Bossert. 1970. Life historical consequences of natural selection. *The American Naturalist* 104:1-24.
- Garland, T., A. F. Bennett, and E. L. Rezende. 2005. Phylogenetic approaches in comparative physiology. *J. Exp. Biol.* 208:3015-3035.
- Garland, T., Jr, and R. Díaz-Uriarte. 1999. Polytomies and phylogenetically Independent Contrasts: examination of the bounded degrees of freedom approach. *Syst. Biol.* 48:547-558.
- Garland, T., Jr, A. W. Dickerman, C. M. Janis, and J. A. Jones. 1993. Phylogenetic analysis of covariance by computer simulation. *Syst. Biol.* 42:265-292.
- Gasser, M., M. Kaiser, D. Berrigan, and S. C. Stearns. 2000. Life-history correlates of evolution under high and low adult mortality. *Evolution* 54:1260-1272.
- Goldschmidt, R. 1940. *The material basis of evolution*. Yale University Press, New Haven, CT.

- Grether, G. F., D. F. Millie, M. J. Bryant, D. N. Reznick, and W. Mayea. 2001. Rain forest canopy cover, resource availability, and life history evolution in guppies. *Ecology* 82:1546-1559.
- Griebeler, E. M., T. Caprano, and K. Böhning-Gaese. 2010. Evolution of avian clutch size along latitudinal gradients: do seasonality, nest predation or breeding season length matter? *J. Evol. Biol.* 23:888-901.
- Grueber, C. E., S. Nakagawa, R. J. Laws, and I. G. Jamieson. 2011. Multimodel inference in ecology and evolution: challenges and solutions. *J. Evol. Biol.* 24:699-711.
- Hansen, T. F. 1997. Stabilizing selection and the comparative analysis of adaptation. *Evolution* 51:1341-1351.
- Hawlena, D., and O. J. Schmitz. 2010. Physiological stress as a fundamental mechanism linking predation to ecosystem functioning. *The American Naturalist* 176:537-556.
- Haynes, J. L. 1995. Standardized classification of Poeciliid development for life-history studies. *Copeia* 1995:147-154.
- Heibo, E., C. Magnhagen, and L. A. Vøllestad. 2005. Latitudinal variation in life-history traits in Eurasian perch. *Ecology* 86:3377-3386.
- Hubble, M. 2003. The ecological significance of body size in tropical wrasses. School of Marine Biology and Aquaculture. James Cook University.
- Hubbs, C. 1971. Competition and isolation mechanisms in the *Gambusia affinis* x *G. heterochir* hybrid swarm. *Bulletin of the Texas Memorial Museum* 19:1-47.

- Hubbs, C., and D. T. Mosier. 1985. Fecundity of *Gambusia gaigei*. *Copeia* 1985:1063-1064.
- James, C., and R. Shine. 1988. Life-history strategies of Australian lizards: a comparison between the tropics and the temperate zone. *Oecologia* 75:307-316.
- Jennions, M. D., and C. D. Kelly. 2002. Geographical variation in male genitalia in *Brachyrhaphis episcopi* (Poeciliidae): is it sexually or naturally selected? *Oikos* 97:79-86.
- Jennions, M. D., and S. R. Telford. 2002. Life-history phenotypes in populations of *Brachyrhaphis episcopi* (Poeciliidae) with different predator communities. *Oecologia* 132:44-50.
- Jeschke, J., and H. Kokko. 2009. The roles of body size and phylogeny in fast and slow life histories. *Evol. Ecol.* 23:867-878.
- Johnson, J. B., and J. C. Bagley. 2011. Ecological drivers of life-history divergence. Pp. 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* 126:142-149.
- Kelly, C. D., J.-G. J. Godin, and G. Abdallah. 2000. Geographical variation in the male intromittent organ of the Trinidadian guppy (*Poecilia reticulata*). *Canadian Journal of Zoology* 78:1674-1680.

- Kembel, S. W., P. D. Cowan, M. R. Helmus, W. K. Cornwell, H. Morlon, D. D. Ackerly, S. P. Blomberg, and C. O. Webb. 2010. Picante: R tools for integrating phylogenies and ecology. *Bioinformatics* 26:1463-1464.
- Langerhans, R. B. 2006. Evolutionary consequences of predation: avoidance, escape, reproduction, and diversification *in* A. M. T. Elewa, ed. *Predation in organisms: a distinct phenomenon*. Springer-Verlag, Heidelberg, Germany.
- Langerhans, R. B., M. E. Gifford, and E. O. Joseph. 2007. Ecological speciation *in Gambusia* fishes. *Evolution* 61:2056-2074.
- Langerhans, R. B., and D. N. Reznick. 2010. Ecology and evolution of swimming performance in fishes: predicting evolution with biomechanics. Pp. 200-248 *in* P. Domenici, and B. G. Kapoor, eds. *Fish Locomotion: an etho-ecological perspective*. Science Publishers, Enfield.
- Lavin, S. R., W. H. Karasov, A. R. Ives, K. M. Middleton, and T. Garland, Jr. 2008. Morphometrics of the avian small intestine compared with that of nonflying mammals: a phylogenetic approach. *Physiol. Biochem. Zool.* 81:526-550.
- Law, R. 1979. Optimal life histories under age-specific predation. *The American Naturalist* 114:399-417.
- Lima, S. L. 1998. Nonlethal effects in the ecology of predator-prey interactions. *Bioscience* 48:25-34.
- Losos, J. B. 2011. Convergence, adaptation, and constraint. *Evolution* 65:1827-1840.

- Losos, J. B., and R. E. Ricklefs. 2009. Adaptation and diversification on islands. *Nature* 457:830-836.
- Lucinda, P. H. F. 2003. Poeciliidae. Pp. 555-581 *in* R. E. Reis, S. O. Kullander, and C. J. Ferraris, eds. Checklist of the Freshwater Fishes of South and Central America. EDIPUCRS, Porto Alegre, Brasil.
- 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* 144:12-24.
- Marsh-Matthews, E., R. Deaton, and M. Brooks. 2010. Survey of matrotrophy in lecithotrophic poeciliids *in* M. C. U. a. H. J. Grier, ed. Viviparous Fishes II, the proceedings of the III International Symposium on Viviparous Fishes. New Life Publications, Homestead, FL.
- Meffe, G. K. 1985. Life history patterns of *Gambusia marshi* (Poeciliidae) from Cuatro Ciénegas, Mexico. *Copeia* 1985:898-905.
- Michod, R. E. 1979. Evolution of life histories in response to age-specific mortality factors. *The American Naturalist* 113:531-550.
- Monteiro, L. R. 1999. Multivariate Regression Models and Geometric Morphometrics: The Search for Causal Factors in the Analysis of Shape. *Syst. Biol.* 48:192-199.
- Oufiero, C. E., G. E. A. Gartner, S. C. Adolph, and T. Garland, Jr. 2011. Latitudinal and climatic variation in body size and dorsal scale counts in *Sceloporus* lizards: a phylogenetic perspective. *Evolution* 65:3590-3607.

- Pagel, M. 1999. Inferring the historical patterns of biological evolution. *Nature* 401:877-884.
- Paine, R. T. 1966. Food web complexity and species diversity. *The American Naturalist* 100:65-75.
- Paradis, E., J. Claude, and K. Strimmer. 2004. APE: Analyses of Phylogenetics and Evolution in R language. *Bioinformatics* 20:289-290.
- Pennings, S. C., and B. R. Silliman. 2005. Linking biogeography and community ecology: latitudinal variation in plant-herbivore interaction strength. *Ecology* 86:2310-2319.
- Pires, M. N., J. Arendt, and D. N. Reznick. 2010. The evolution of placentas and superfetation in the fish genus *Poecilia* (Cyprinodontiformes: Poeciliidae: subgenera *Micropoecilia* and *Acanthophaelus*). *Biol. J. Linn. Soc.* 99:784-796.
- Pires, M. N., A. Banet, B. J. A. Pollux, and D. N. Reznick. 2011a. Variation and evolution of reproductive strategies. Pp. 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., R. D. Bassar, K. E. McBride, J. U. Regus, T. Garland, Jr, and D. N. Reznick. 2011b. Why do placentas evolve? An evaluation of the life-history facilitation hypothesis in the fish genus *Poeciliopsis*. *Funct. Ecol.* 25:757-768.

- Poff, N. L., J. D. Allan, M. B. Bain, J. R. Karr, K. L. Prestegard, B. D. Richter, R. E. Sparks, and J. C. Stromberg. 1997. The Natural Flow Regime. *Bioscience* 47:769-784.
- 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, Evolution, and Systematics* 40:271-289.
- Ponce de León, J. L., R. Rodríguez, M. Acosta, and M. C. Uribe. 2011. Egg size and its relationship with fecundity, newborn length and female size in Cuban poeciliid fishes (Teleostei: Cyprinodontiformes). *Ecol. Freshwat. Fish* 20:243-250.
- R Development Core Team. 2011. R: A language and environment for statistical computing. R Foundation for Statistical Computing, Vienna.
- Rezende, E. L., F. Bozinovic, and T. Garland, Jr. 2004. Climatic adaptation and the evolution of basal and maximum rates of metabolism in rodents. *Evolution* 58:1361-1374.
- Reznick, D. N., H. Bryga, and J. A. Endler. 1990. Experimentally induced life-history evolution in a natural population. *Nature* 346:357-359.
- 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. N., and D. B. Miles. 1989. Poeciliid life history patterns. Pp. 373-377
in G. K. Meffe, and F. F. Snelson, eds. Ecology and evolution of livebearing
fishes (Poeciliidae). Prentice Hall, Englewood Cliffs, NJ.
- Reznick, D. N., F. H. Rodd, and M. Cardenas. 1996. Life-history evolution in
guppies (*Poecilia reticulata*: Poeciliidae). IV. Parallelism in life-history
phenotypes. *The American Naturalist* 147:319-338.
- Riesch, R., A. Oranth, J. Dzienko, N. Karau, A. Schießl, S. Stadler, A. Wigh, C.
Zimmer, L. Arias-Rodriguez, I. Schlupp, and M. Plath. 2010a. Extreme
habitats are not refuges: poeciliids suffer from increased aerial predation risk
in sulphidic southern Mexican habitats. *Biol. J. Linn. Soc.* 101:417-426.
- Riesch, R., M. Plath, F. García de León, and I. Schlupp. 2010b. Convergent life-
history shifts: toxic environments result in big babies in two clades of
poeciliids. *Naturwissenschaften* 97:133-141.
- Roff, D. A. 1992. *The Evolution of Life Histories: Theory and Analysis*. Chapman
and Hall, New York, NY.
- Roff, D. A. 2002. *Life History Evolution*. Sinauer Associates Inc., Sunderland, MA.
- Rosen, D. E., and R. M. Bailey. 1963. The Poeciliid Fishes (Cyprinodontiformes),
their structure, zoogeography, and systematics. *Bull. Am. Mus. Nat. Hist. N. Y.*
126:1-176.
- Sabaj-Pérez, M. H. 2010. Standard symbolic codes for institutional resource
collections in herpetology and ichthyology: an Online Reference. American
Society of Ichthyologists and Herpetologists, Washington D. C.

- Sabo, J. L., and D. M. Post. 2008. Quantifying periodic, stochastic, and catastrophic environmental variation. *Ecol. Monogr.* 78:19-40.
- Sæther, B.-E., and Ø. Bakke. 2000. Avian life history variation and contribution of demographic traits to the population growth rate. *Ecology* 81:642-653.
- Schaffer, W. M. 1974. Selection for optimal life histories: the effects of age structure. *Ecology* 55:291-303.
- Scrimshaw, N. S. 1945. Embryonic development in Poeciliid fishes. *The Biological Bulletin* 88:233-246.
- Sharpe, D. M. T., and A. P. Hendry. 2009. Life history change in commercially exploited fish stocks: an analysis of trends across studies. *Evolutionary Applications* 2:260-275.
- Sibly, R. M., and P. Calow. 1986. *Physiological Ecology of Animals: An Evolutionary Approach*. Blackwell Scientific Publications, Oxford.
- Sih, A., P. Crowley, M. McPeck, J. Petranka, and K. Strohmeier. 1985. Predation, competition, and prey communities: a review of field experiments. *Annu. Rev. Ecol. Syst.* 16:269-311.
- Slinker, B. K., and S. A. Glantz. 1985. Multiple regression for physiological data analysis: the problem of multicollinearity. *American Journal of Physiology - Regulatory, Integrative and Comparative Physiology* 249:R1-R12.
- Smith, C. C., and S. D. Fretwell. 1974. The Optimal balance between size and number of offspring. *The American Naturalist* 108:499-506.

- Sokolovska, N., L. Rowe, and F. Johansson. 2000. Fitness and body size in mature odonates. *Ecol. Entomol.* 25:239-248.
- Stanley, S. M. 1979. *Macroevolution: Pattern and Process*. W. H. Freeman and Co., San Francisco.
- Stearns, S. C. 1992. *The Evolution of Life Histories*. Oxford University Press, Oxford, UK.
- Tabachnick, B. G., and L. S. Fidell. 2001. *Using Multivariate Statistics*. Allyn & Bacon.
- Tinkle, D. W., and R. E. Ballinger. 1972. *Sceloporus undulatus*: a study of the intraspecific comparative demography of a lizard. *Ecology* 53:570-584.
- Traynor, R. E., and P. J. Mayhew. 2005. A comparative study of body size and clutch size across the parasitoid Hymenoptera. *Oikos* 109:305-316.
- Turner, C. L. 1940. Superfetation in viviparous Cyprinodont fishes. *Copeia* 1940:88-91.
- Vamosi, S. M. 2005. On the role of enemies in divergence and diversification of prey: a review and synthesis. *Canadian Journal of Zoology* 83:894-910.
- Van Valen, L. 1974. A natural model for the origin of some higher taxa. *J. Herpetol.* 8:109-121.
- Vila-Gispert, A., R. Moreno-Amich, and E. García-Berthou. 2002. Gradients of life-history variation: an intercontinental comparison of fishes. *Rev. Fish Biol. Fish.* 12:417-427.

- Walsh, M. R., and D. M. Post. 2011. Interpopulation variation in a fish predator drives evolutionary divergence in prey in lakes. *Proceedings of the Royal Society B: Biological Sciences* 278:2628-2637.
- Walsh, M. R., and D. N. Reznick. 2009. Phenotypic diversification across an environmental gradient: a role for predators and resource availability on the evolution of life histories. *Evolution* 63:3201-3213.
- Watson, D. M. S., N. W. Timofeeff-Ressovsky, E. J. Salisbury, W. B. Turrill, T. J. Jenkin, R. R. Gates, R. A. Fisher, C. Diver, G. D. H. Carpenter, J. B. S. Haldane, E. W. MacBride, and R. N. Salaman. 1936. A discussion on the present state of the Theory of Natural Selection. *Proceedings of the Royal Society of London. Series B - Biological Sciences* 121:43-73.
- Wellborn, G. A. 1994. Size-biased predation and prey life histories: a comparative study of freshwater amphipod populations. *Ecology* 75:2104-2117.
- Wetzel, R. G., and G. E. Likens. 2000. *Limnological analyses*. Springer Verlag, New York.
- Williams, G. C. 1966. Natural selection, the costs of reproduction, and a refinement of Lack's Principle. *The American Naturalist* 100:687-690.
- Wohl, E., and D. M. Merritt. 2008. Reach-scale channel geometry of mountain streams. *Geomorphology* 93:168-185.
- Wourms, J. P. 1981. Viviparity: The maternal-fetal relationship in fishes. *Am. Zool.* 21:473-515.

Wourms, J. P., B. D. Grove, and J. Lombardi. 1988. The maternal-embryonic relationship in viviparous fishes. Pp. 1-135 *in* W. S. Hoar, and D. J. Randall, eds. Fish Physiology. Academic Press, New York.

Table 2.1. Environmental characteristics and average life histories of *Gambusia* samples. Predation (P) and Flow (F) levels were either low (L) or high (H). Sample size (n) for each collection corresponds to the total number of individuals analyzed for estimating SL_{min} over the number of individuals used in further analyses. Embryo number, and embryo and ovary dry weights are estimated values at birth for the average SL female.

ID	Species	Collection ^a	P	F	Latitude	n	SL (mm)	Fem. wet w. (g)	SL_{min} (mm)	# emb.	Emb. weight (mg)	Ovary weight (mg)	MI
1	<i>G. alvarezi</i>	UMMZ 211110	L	L	27.010	30/16	28.79	0.507	25.13	9.0	2.51	24.93	0.69
2	<i>G. atrora</i>	AMNH 40812, AMNH 77929	H	H	21.499	35/28	27.74	0.289	22.49	4.8	2.68	13.59	0.93 *
3	<i>G. atrora</i>	UMMZ 169499, UMMZ210724	H	H	21.438	59/41	25.29	0.200	19.86	3.5	2.59	10.07	0.72
4	<i>G. aurata</i>	AMNH75821, RBL	L	L	22.765	18/13	24.05	0.232	18.20	4.9	1.08	4.62	0.81
5	<i>G. baracoana</i>	USNM 204442	H	L	20.350	30/12	28.25	0.317	24.13	9.7	1.12	11.58	0.75
6	<i>G. clarkhubbsi</i>	TCWC 11880-09, TCWC 11882-07, TCWC 11887-08	L	H	29.369	34/24	27.58	0.312	20.40	3.9	2.03	9.43	0.45
7	<i>G. geiseri</i>	TNHC 9132, TNHC 9146	L	L	29.876	70/55	25.21	0.212	17.86	6.8	0.71	4.87	0.54
8	<i>G. hurtadoi</i>	UMMZ 196737, UMMZ 211112	H	H	27.030	59/28	24.74	0.286	19.91 ^b	7.3	1.75	14.00	0.74
9	<i>G. krumholzi</i>	KU 7341	H	L	28.412	30/23	37.97	0.835	29.21	17.0	3.19	60.99	0.55
10	<i>G. lemaîtrei</i>	UIST (uncatalogued)	H	L	10.734	21/13	22.79	0.223	18.47	8.0	1.21	9.73	0.89
11	<i>G. luma</i>	FMNH 87628, FMNH 94163, UMMZ 197235, UMMZ 197258	H	H	16.169	69/29	30.06	0.466	23.93	5.5	1.65	9.26	0.89(*)
12	<i>G. manni</i>	ANSP 103450, ANSP 103452, RBL	H	L	25.047	75/48	27.12	0.322	20.49	3.8	2.55	11.16	0.75
13	<i>G. hubbsi</i>	UMMZ 202727	H	L	25.700	21/16	27.02	0.362	19.98 ^b	8.1	0.88	8.02	0.56
14	<i>G. melaleura</i>	USNM 205559	L	H	18.300	26/21	33.02	0.712	24.01	6.3	2.11	14.64	0.81
15	<i>G. nicaraguensis</i>	UMMZ 199657, UMMZ 199689	H	H	15.358	40/30	28.75	0.418	20.41 ^b	15.4	0.99	19.38	0.87
16	<i>G. oligosticta</i>	UMMZ 190129, RBL	H	L	17.929	38/31	22.48	0.189	17.84	7.7	0.92	7.78	0.48
17	<i>G. punctata</i>	AMNH 96308	H	H	22.699	20/14	40.24	1.222	33.61	7.6	3.32	29.14	0.84
18	<i>G. punctata</i>	AMNH 96320	H	H	22.721	20/14	46.30	1.685	33.49	7.7	4.43	39.61	0.63

ID Species	Collection ^a	P	F	Latitude	n	SL (mm)	Fem. wet w. (g)	SL _{min} (mm)	# emb.	Emb. weight (mg)	Ovary weight (mg)	MI
19 <i>G. punctata</i>	USNM 203197, USNM 203198	H	H	21.117	60/50	41.79	1.290	32.04	11.7	3.24	38.27	0.78
20 <i>G. rhizophorae</i>	TCWC 2577-02, TCWC 8671-01, UMMZ 213650, RBL	H	L	24.857	39/15	29.13	0.516	18.32	3.7	2.10	6.71	1.24 ^(*)
21 <i>G. sexradiata</i>	UMMZ 210795, UMMZ 196655	H	L	18.024	58/39	29.17	0.590	24.63	10.7	1.16	13.81	0.73
22 <i>G. vittata</i>	AMNH 75838	H	H	21.338	20/15	31.63	0.405	28.50	5.4	3.26	19.26	0.96 ^(*)
23 <i>G. vittata</i>	UMMZ 192503	H	H	23.324	27/24	26.44	0.255	20.36	4.6	1.71	7.79	0.64
24 <i>G. vittata</i>	UMMZ 97513	H	H	21.989	30/29	23.24	0.191	19.48 ^b	9.9	0.92	10.60	0.65
25 <i>G. vittata</i>	UMMZ 97517	H	H	21.064	30/25	27.98	0.285	22.95	12.4	1.13	18.04	0.81
26 <i>G. wrayi</i>	USNM 205574, UF 25054	H	L	18.024	55/42	37.50	0.888	27.80	13.6	1.65	23.74	0.70
	Minimum			10.734	-	22.48	0.189	17.84	3.5	0.71	4.62	0.45
	Maximum			29.876	-	46.30	1.685	33.61	17.0	4.43	60.99	0.89
	Times-Fold			2.783	-	2.06	8.909	1.88	4.9	6.22	13.20	1.98

SL= standard length. SL_{min}=minimum size at maturity. MI= matrotrophy index.

^a Institutional abbreviations follow Sabaj-Perez (2010), except for RBL, a personal collection of one of the authors. Collections with multiple catalog numbers correspond to data from nearby collections (same species and environmental regimes) that were merged to increase sample size. Further details in Table 1.1 and Appendix 1.1.

^b These are the only collections without juvenile females smaller than the minimum size at maturity. These SL_{min} values were rather low compared with other *Gambusia* species, suggesting an overestimation of size at maturity was unlikely. P-values of MI: 0.1 > (*) > 0.05 > *.

Table 2. 2. Principal Component Analysis of log-transformed life history traits.

	PC 1	PC 2	PC 3	PC 4	PC 5
% Variance	69.4	24.7	4.3	1.4	0.1
Cumul. % Variance	69.2	94.2	98.5	99.9	100.0
Eigenvalue	3.5	1.2	0.2	0.1	0.0
Loadings					
Standard Length	-0.505	0.070	0.629	0.584	0.065
Size at Maturity	-0.519	0.018	0.320	-0.792	-0.028
Number embryos	-0.245	-0.796	-0.148	0.101	-0.523
Embryo dry weight	-0.394	0.581	-0.433	0.116	-0.554
Ovary dry weight	-0.510	-0.154	-0.542	0.089	0.644

Table 2.3. Phylogenetic signal (K) of dependent and independent variables. Dependent variables are the estimated values for the hypothetical mean-size female. β_{size} =allometric coefficient. K was calculated with the original tree (K_M) with branch lengths proportional to rate of nucleotide substitution, and with a tree with arbitrary constant branch lengths (all =1, K_C). *P*-values indicate the probability that phylogenetic signal is present based on a randomization test of the mean squared error (Blomberg et al., 2003).

Variable	K_M	<i>P</i>	K_C	<i>P</i>
Predation	0.138	0.077	0.361	0.334
Flow	0.195	0.006	0.406	0.118
Latitude	0.824	0.000	1.307	0.000
Standard Length	0.126	0.058	0.515	0.007
Female wet weight	0.183	0.011	0.649	0.001
Minimum size at maturity	0.091	0.138	0.398	0.100
β_{size} number of embryos	0.059	0.138	0.604	0.691
Number of embryos	0.048	0.707	0.282	0.808
β_{size} mean emb. dry w.	0.103	0.087	0.449	0.063
Mean embryo dry weight	0.092	0.110	0.437	0.062
β_{size} ovary dry w.	0.103	0.134	0.764	0.060
Ovary dry weight	0.077	0.262	0.379	0.350
Matrotrophy Index	0.087	0.276	0.451	0.210
Principal Component 1	0.091	0.137	0.395	0.092
Principal Component 2	0.053	0.579	0.303	0.551

Table 2.4. Regressions, showing best-supported model and average model for each trait. S=log SL. P=Predation. F=Flow. L=Latitude. Five adaptive models were tested for each dependent variable: M~S, M~S+P, M~S+F, M~S+L, M~S+P+F+L, where S= log SL, P=Predation, F=Flow, and L=Latitude. When log SL or PC scores were the dependent variables, S was not used as a covariate. The three models of character evolution tested were ordinary least-squares (OLS, non-phylogenetic), Pagel's (RegPagel), and Ornstein–Uhlenbeck (RegOU). Branch-length transformations were either proportional to molecular distances (M) or constant (C) branch lengths. The value w is the Akaike weight for the best model or the cumulative weight (within $\Delta AIC_c=4$) for the average model of each trait. The value d is the phylogenetic signal for RegOU models. SL=standard length, W_{fem} =female wet weight. SL_{min} =minimum size at maturity. β_{size} =allometric coefficient. N_{emb} =number of embryos. W_{emb} =mean embryo weight. W_{ovary} =ovary dry weight. MI=matrotrophy index. PC = principal component. Full list and further details of top-set models in Supplementary Materials.

Trait	Model	Character Evolution	Tree	w	d	Coefficient ± SE				p-value		
						log SL	Predation	Flow	Latitude			
log SL	P	RegOU	M	23.5	0.039				0.107 ± 0.086	0.234		
	Average	-	-	84.7	-				0.039 ± 0.070		0.011 ± 0.042	<0.001 ± 0.004
log W _{fem}	P	RegOU	M	29.4	0.159				0.404 ± 0.260	0.140		
	Average	-	-	82.3	-				0.153 ± 0.242		-0.013 ± 0.114	<0.001 ± 0.014
log SL _{min}	P	RegOU	M	22.3	0.007				0.114 ± 0.093	0.240		
	Average	-	-	90.8	-				0.044 ± 0.080		0.029 ± 0.061	<0.001 ± 0.004
β _{size} N _{emb}	S	OLS	-	25.2	-	2.044 ± 0.761	0.013					
	Average	-	-	76.1	-	1.691 ± 0.758			0.162 ± 0.309		-0.004 ± 0.077	-0.004 ± 0.015
log N _{emb}	S	OLS	-	20.1	-	0.832 ± 0.462	0.086					
	Average	-	-	66.9	-	0.634 ± 0.444			0.017 ± 0.086		-0.029 ± 0.098	-0.007 ± 0.015
β _{size} W _{emb}	S	OLS	-	23.3	-	0.902 ± 0.520	0.097					
	Average	-	-	70.3	-	0.622 ± 0.528			0.009 ± 0.074		0.056 ± 0.142	<0.001 ± 0.005
log W _{emb}	S	OLS	-	16.4	-	1.828 ± 0.394	<0.001					
	Average	-	-	78.3	-	1.637 ± 0.432			0.001 ± 0.039		0.057 ± 0.121	0.003 ± 0.010
β _{size} W _{ovary}	S+P	OLS	-	45.5	-	2.955 ± 0.612	<0.001		0.824 ± 0.292	0.010		
	Average	-	-	80.5	-	2.397 ± 0.756			0.659 ± 0.299		<0.001 ± <0.001	<0.001 ± <0.001
log W _{ovary}	S	OLS	-	25.4	-	2.739 ± 0.402	<0.001					
	Average	-	-	76.3	-	2.402 ± 0.494			0.087 ± 0.165		0.003 ± 0.043	-0.001 ± 0.006
MI	S+L	OLS	-	29.5	-	0.085 ± 0.169	0.622					-0.014 ± 0.007
	Average	-	-	78.3	-	0.068 ± 0.167			0.009 ± 0.038		0.002 ± 0.018	-0.006 ± 0.008
PC 1	P	RegOU	M	30.6	0.015				1.428 ± 0.87	0.118		
	Average	-	-	76.1	-				0.615 ± 0.85		0.169 ± 0.479	-0.002 ± 0.026
PC 2	F	OLS	-	20.6	-							-0.073 ± 0.048
	Average	-	-	84.9	-				0.038 ± 0.229		-0.291 ± 0.420	-0.029 ± 0.046

Figure 2.1. Representation of the Principal Component 2, showing the relationship between the two variables with loadings > 0.32 in that axis (following the criterium of Tabachnick and Fidell, 2001). Values were estimated using Burnaby back projection, which projects them in a plane orthogonal Principal Components 1 (Berner, 2011). It is similar to estimating the residuals of PC1, except for producing scores of values in their original scale.

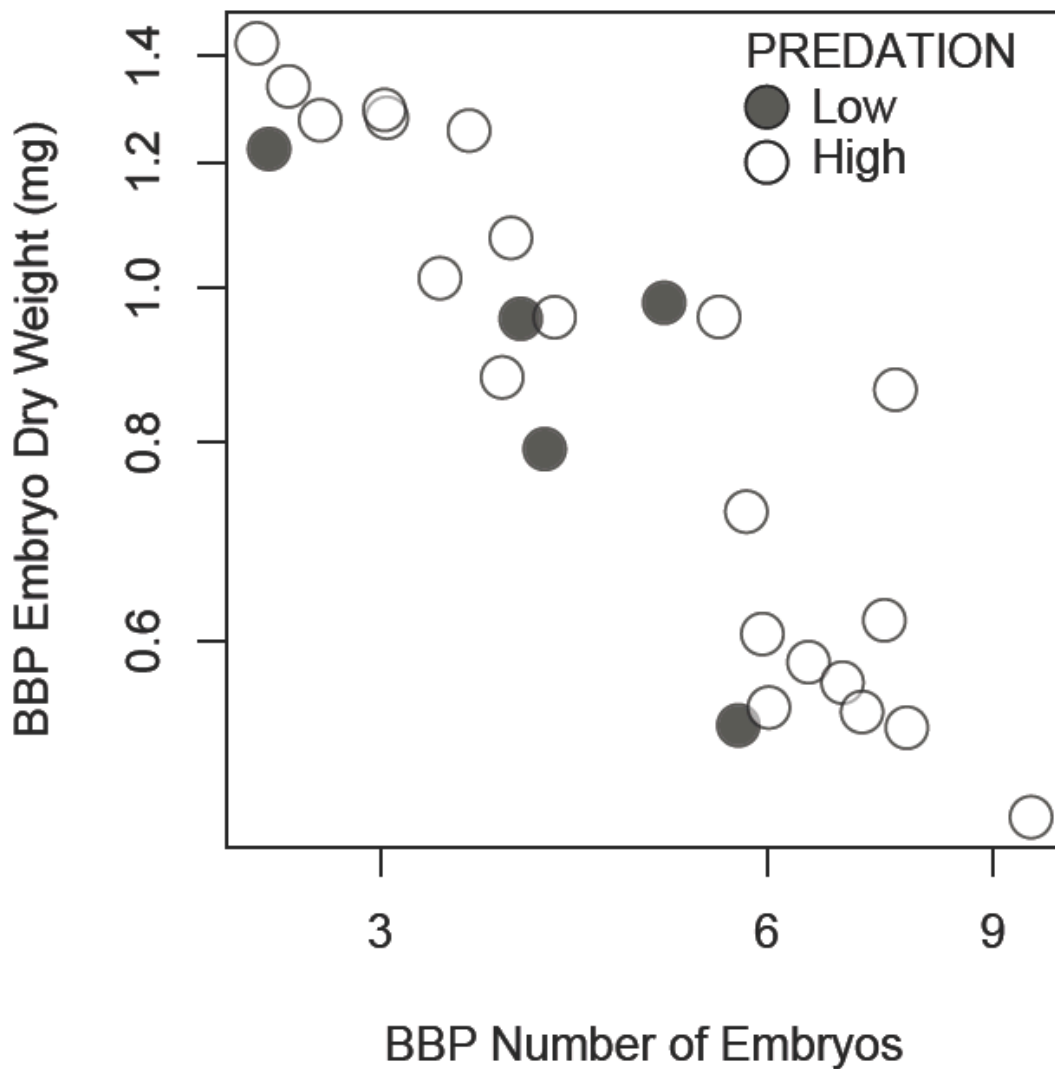


Figure 2.2. Association between mean values of log Standard Length and mean values of life history traits. Original values on left side, phylogenetic independent contrasts (PIC) on right side. Best-fit lines in black. Bottom-left panel with gray line that indicates the threshold value for Matrotrophy Index (MI=0.7). Axes in logarithmic scale. Histograms beside boxes have bar widths proportional to intervals in linear scale. PIC were calculated with original branch length tree.

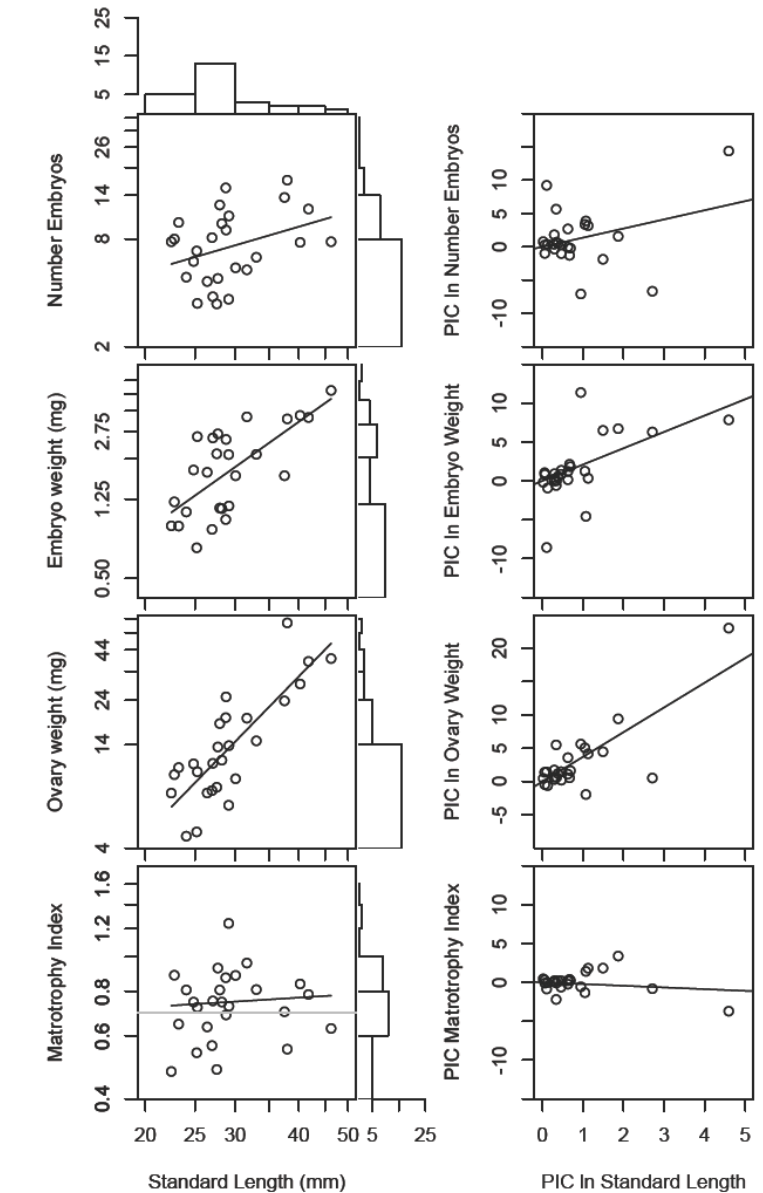
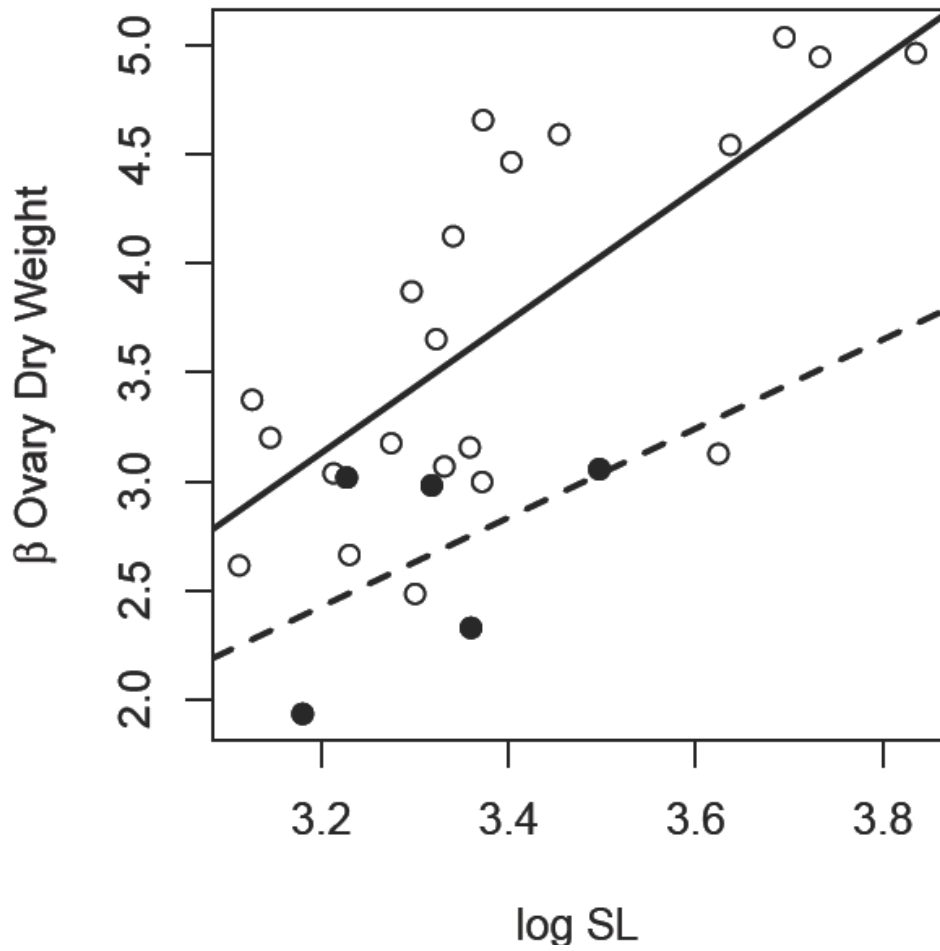


Figure 2.3. Relationship between predation and the allometric coefficient of ovary dry weight for each collection. Filled dots = Low Predation, empty dots = High Predation.



Appendix 2.1. Estimated intercepts (l), allometric coefficients (β_{size}), and stage of development coefficients (β_{stage}) for each trait and each collection. Values were obtained by model averaging the general model ($\log \text{trait} = l + \beta_{\text{size}} (\log \text{SL}) + \beta_{\text{stage}}$ (stage) + ϵ) and its nested models. ID numbers match Table 2.1. Values of $\beta_{\text{stage}} \pm \text{SE}$ are multiplied by $10e2$. Bold indicates either a significant deviation from isometry ($\beta_{\text{size}} = 3$) or β_{stage} values significantly different from zero.

ID Species	log Number of embryos			log Mean embryo dry weight			log Ovary dry weight		
	$l \pm \text{SE}$	$\beta_{\text{size}} \pm \text{SE}$	$\beta_{\text{stage}} \pm \text{SE}$	$l \pm \text{SE}$	$\beta_{\text{size}} \pm \text{SE}$	$\beta_{\text{stage}} \pm \text{SE}$	$l \pm \text{SE}$	$\beta_{\text{size}} \pm \text{SE}$	$\beta_{\text{stage}} \pm \text{SE}$
1 <i>G. alvarezi</i>	-6.40±2.37	2.63±0.70	-0.45±0.38	2.22±1.68	-0.27±0.50	-0.80±0.32	-10.95±1.61	2.33±0.48	-1.12±0.25
2 <i>G. atrora</i>	-8.94±0.95	3.29±0.29	-0.83±0.34	0.39±0.66	0.19±0.20	-0.08±0.17	-15.88±0.69	3.65±0.21	-1.11±0.23
3 <i>G. atrora</i>	-6.34±0.96	2.36±0.30	-0.06±0.25	0.99±0.53	0.09±0.16	-0.68±0.21	-12.92±0.81	2.66±0.25	-0.56±0.31
4 <i>G. aurata</i>	-5.34±2.37	2.34±0.73	-1.05±1.11	2.23±1.82	-0.65±0.57	-0.18±0.62	-10.39±1.33	1.93±0.41	-2.28±0.64
5 <i>G. baracoana</i>	-9.66±3.47	3.58±1.05	-0.07±0.51	-0.67±1.87	0.33±0.58	-0.66±0.42	-17.91±3.31	4.13±1.02	-0.67±0.65
6 <i>G. clarkhubbsi</i>	-4.88±1.66	1.88±0.50	-0.02±0.36	-2.44±1.10	1.18±0.33	-1.51±0.32	-14.11±0.99	3.07±0.30	-1.45±0.30
7 <i>G. geiseri</i>	-4.02±0.98	1.77±0.32	0.47±0.44	-3.71±0.71	1.31±0.23	-1.70±0.30	-14.27±0.92	3.02±0.30	-1.58±0.39
8 <i>G. hurtadoi</i>	-10.16±1.67	3.76±0.51	0.15±0.33	-0.51±1.06	0.41±0.32	-0.48±0.24	-17.58±1.28	4.19±0.39	-0.29±0.30
9 <i>G. krumholzi</i>	-11.40±2.03	3.86±0.55	0.38±0.56	-0.48±1.29	0.60±0.35	-1.05±0.35	-19.23±1.57	4.54±0.43	-0.19±0.39
10 <i>G. femaltrei</i>	-4.30±2.53	2.15±0.80	-0.70±0.73	-3.27±1.59	1.12±0.51	-0.05±0.32	-14.69±1.97	3.38±0.62	-1.00±0.64
11 <i>G. luma</i>	-10.48±1.78	3.71±0.53	-0.89±0.50	-0.69±1.10	0.37±0.32	-0.17±0.24	-19.19±1.70	4.47±0.50	-1.38±0.44
12 <i>G. manni</i>	-7.98±1.04	2.73±0.31	0.61±0.51	1.23±0.47	-0.01±0.14	-0.53±0.33	-12.74±0.92	2.48±0.28	0.09±0.31
13 <i>G. hubbsi</i>	-7.67±1.63	3.28±0.55	-2.10±1.04	-0.97±1.03	0.54±0.35	-1.84±0.58	-15.76±1.52	3.87±0.51	-3.66±0.90
14 <i>G. melapleura</i>	-7.22±1.44	2.68±0.41	-0.65±0.53	-0.47±0.88	0.41±0.25	-0.46±0.30	-14.40±1.35	3.06±0.39	-1.03±0.50
15 <i>G. nicaraguensis</i>	-4.74±1.27	2.40±0.37	-1.18±0.79	-1.92±0.61	0.59±0.18	-0.19±0.30	-14.13±0.90	3.16±0.27	-0.83±0.57
16 <i>G. oligosticta</i>	-4.48±1.33	2.04±0.39	0.36±0.71	-1.03±1.04	0.47±0.30	-1.04±0.63	-12.89±1.09	2.61±0.33	-0.21±0.56
17 <i>G. punctata</i>	-11.69±2.98	3.86±0.82	-1.06±0.64	-2.69±2.17	1.13±0.60	-0.60±0.43	-21.46±1.99	5.04±0.55	-1.39±0.40
18 <i>G. punctata</i>	-8.94±4.99	2.83±1.29	0.24±0.53	-3.12±2.90	1.31±0.74	-0.80±0.39	-22.08±2.63	4.97±0.68	-0.39±0.37
19 <i>G. punctata</i>	-12.42±1.92	4.00±0.51	-0.11±0.34	-2.22±1.82	0.93±0.48	-0.19±0.33	-21.53±1.11	4.95±0.29	-0.42±0.28
20 <i>G. rhizophorae</i>	-2.84±3.05	1.26±0.93	-0.23±0.99	-2.39±0.98	0.95±0.30	-0.11±0.35	-14.03±2.22	3.00±0.69	-2.16±1.12
21 <i>G. sexradiata</i>	-12.03±2.20	4.30±0.65	-0.19±0.35	-0.21±1.00	0.20±0.30	-0.66±0.20	-19.55±1.89	4.66±0.56	-0.90±0.38
22 <i>G. vittata</i>	-9.30±2.31	3.43±0.66	-1.73±0.47	-2.83±1.11	1.15±0.32	0.11±0.19	-19.12±1.94	4.59±0.55	-1.39±0.39
23 <i>G. vittata</i>	-3.59±1.62	1.49±0.51	0.48±0.51	-1.06±0.50	0.66±0.16	-1.13±0.16	-14.62±1.24	3.18±0.39	-1.27±0.40
24 <i>G. vittata</i>	-7.83±1.76	3.16±0.54	0.37±0.54	0.08±0.61	0.08±0.19	-0.85±0.24	-14.52±1.63	3.20±0.50	-0.19±0.45
25 <i>G. vittata</i>	-4.64±0.84	2.22±0.25	-0.48±0.29	-3.76±0.95	1.23±0.29	-0.44±0.32	-14.09±1.14	3.07±0.34	-0.31±0.35
26 <i>G. wrayi</i>	-7.28±0.97	2.75±0.27	-0.12±0.22	0.05±0.78	0.23±0.22	-0.77±0.19	-14.63±0.88	3.13±0.25	-0.89±0.23

Appendix 2.2. Candidate set ($\Delta AIC_c=4$) of regressions for size variables and each life history trait. Five adaptive models were tested for each dependent variable: M~S, M~S+P, M~S+F, M~S+L, M~S+P+F+L, where S= log SL, P=Predation, F=Flow, and L=Latitude. The same models without S as the covariate were tested when Standard Length, female weight, size at maturity, and Principal Components as dependent variables. The three models of character evolution tested were ordinary least-squares (OLS, non-phylogenetic), Pagel's (RegPagel), and Ornstein-Uhlenbeck (RegOU). Branch-length transformations were either proportional to molecular distances (M) or constant (C) branch lengths. k=number of parameters, lnML=log Maximal Likelihood, AIC_c=small sample corrected Akaike Information Criterion, and w=Akaike weights. Evidence Ratio (ER) indicates the number of times the most-supported model of the set is better supported than each row's model. Phylogenetic signal was λ for RegPagel, and d for RegOU.

Model	Character Evolution	Tree k	lnML	AIC _c	w	λ or d	ER	Coefficients \pm SE (P-value)						
								log SL	Predation	Flow	Latitude			
P	RegOU	M	4	10.9	-12.0	23.5	0.039	1.0	log Standard length					
F	RegOU	M	4	10.3	-10.7	12.6	0.014	1.9		0.107 \pm 0.086	0.233			
L	RegOU	M	4	10.2	-10.5	11.6	0.022	2.0				0.028 \pm 0.077	0.716	
P	RegOU	C	4	10.0	-10.1	9.5	0.620	2.5		0.080 \pm 0.078	0.325		0.000 \pm 0.008	0.985
F	RegOU	C	4	9.8	-9.7	7.6	0.558	3.1				0.045 \pm 0.068	0.515	
P	RegPagel	C	4	9.7	-9.6	7.2	0.831	3.3		0.089 \pm 0.078	0.268			
F	RegPagel	C	4	9.6	-9.4	6.6	0.727	3.6				0.068 \pm 0.065	0.313	
L	RegOU	C	4	9.6	-9.3	6.1	0.640	3.9				0.004 \pm 0.009	0.681	
L	RegPagel	C	4	9.1	-8.4	3.9	0.887	6.0				0.004 \pm 0.009	0.687	
log Female weight														
P	RegOU	M	4	-18.1	46.1	29.4	0.159	1.0		0.404 \pm 0.260	0.139			
F	RegOU	M	4	-19.1	48.1	11.3	0.118	2.6				-0.126 \pm 0.244	0.613	
L	RegOU	M	4	-19.1	48.1	11.1	0.087	2.7					-0.008 \pm 0.027	0.770
P	RegOU	C	4	-19.3	48.4	9.4	0.787	3.1		0.200 \pm 0.234	0.404			
P	RegPagel	C	4	-19.4	48.8	7.8	1.045	3.8		0.197 \pm 0.222	0.386			
L	RegOU	C	4	-19.6	49.1	6.7	0.813	4.4					0.008 \pm 0.029	0.778
F	RegOU	C	4	-19.6	49.1	6.6	0.758	4.5				0.030 \pm 0.203	0.885	
L	RegPagel	C	4	-19.8	49.6	5.2	1.045	5.7					0.012 \pm 0.031	0.699
F	RegPagel	C	4	-19.9	49.7	4.9	1.045	6.0				-0.021 \pm 0.196	0.914	
log Size at maturity														

Model	Character Evolution	Tree	k	lnML	AICc	w	λ or d	ER	Coefficients \pm SE (<i>P</i> -value)				
									log SL	Predation	Flow	Latitude	
P	RegOU	M	4	8.7	-7.5	22.3	0.007	1.0	0.114 \pm 0.093	0.240			
F	RegOU	M	4	8.2	-6.6	14.0	0.001	1.6			0.058 \pm 0.081	0.480	
F	OLS	-	3	6.7	-6.4	12.8	-	1.7			0.106 \pm 0.076	0.179	
L	RegOU	M	4	8.0	-6.1	11.2	0.005	2.0				-0.003 \pm 0.009	0.765
P	OLS	-	3	6.3	-5.6	8.5	-	2.6	0.105 \pm 0.098	0.297			
P	RegOU	C	4	7.2	-4.6	5.1	0.328	4.4	0.104 \pm 0.092	0.276			
F	RegOU	C	4	7.2	-4.4	4.8	0.224	4.7			0.076 \pm 0.077	0.339	
L	OLS	-	3	5.7	-4.4	4.7	-	4.7				-0.001 \pm 0.009	0.917
F	RegPage1	C	4	6.9	-3.9	3.7	0.260	6.0			0.097 \pm 0.073	0.205	
P	RegPage1	C	4	6.9	-3.9	3.7	0.358	6.0	0.131 \pm 0.093	0.178			
Allometric coefficient of number of embryos													
S	OLS	-	3	-27.6	62.3	25.2	-	1.0	2.044 \pm 0.761	0.013			
S+P	OLS	-	4	-26.4	62.7	21.1	-	1.2	1.870 \pm 0.750	0.021	0.540 \pm 0.357	0.146	
S+L	OLS	-	4	-27.0	63.9	11.2	-	2.3	2.087 \pm 0.761	0.012		-0.033 \pm 0.032	0.309
S+F	OLS	-	4	-27.6	65.1	6.3	-	4.0	2.081 \pm 0.798	0.016		-0.061 \pm 0.300	0.841
S	RegOU	C	4	-27.6	65.1	6.2	<0.001	4.1	2.044 \pm 0.761	0.016			
S	RegOU	M	4	-27.6	65.2	6.1	<0.001	4.1	2.044 \pm 0.762	0.016			
S+P	RegOU	C	5	-26.4	65.8	4.5	<0.001	5.6	1.870 \pm 0.750	0.024	0.540 \pm 0.357	0.150	
S+P	RegOU	M	5	-26.4	65.8	4.4	<0.001	5.7	1.870 \pm 0.752	0.024	0.540 \pm 0.358	0.150	
log Number of embryos													
S	OLS	-	3	-14.7	36.4	20.1	-	1.0	0.832 \pm 0.462	0.086			
S+L	OLS	-	4	-13.5	36.8	16.2	-	1.2	0.868 \pm 0.452	0.068		-0.028 \pm 0.019	0.153
S+F	OLS	-	4	-13.7	37.3	12.6	-	1.6	0.972 \pm 0.468	0.050		-0.231 \pm 0.176	0.204
S+P	OLS	-	4	-14.1	38.2	8.2	-	2.5	0.763 \pm 0.469	0.118	0.213 \pm 0.223	0.351	
S	RegOU	C	4	-14.7	39.2	4.9	<0.001	4.1	0.832 \pm 0.462	0.090			
S	RegOU	M	4	-14.7	39.2	4.9	<0.001	4.1	0.832 \pm 0.463	0.090			
S+L	RegOU	C	5	-13.5	39.9	3.4	<0.001	5.9	0.868 \pm 0.452	0.073		-0.028 \pm 0.019	0.158
S+L	RegOU	M	5	-13.5	39.9	3.4	<0.001	5.9	0.869 \pm 0.454	0.073		-0.028 \pm 0.019	0.157
Allometric coefficient of embryo dry weight													

Model	Character Evolution	Tree k	lnML	AICc	w	λ or d	ER	Coefficients \pm SE (P-value)					
								log SL	Predation	Flow	Latitude		
S	OLS	-	3	-17.7	42.5	23.3	-	1.0	0.902 \pm 0.520	0.097			
S+F	OLS	-	4	-16.9	43.8	12.0	-	1.9	0.761 \pm 0.530	0.166	0.233 \pm 0.200	0.256	
S	RegPagel	M	4	-17.3	44.5	8.6	0.492	2.7	0.726 \pm 0.534	0.191			
S	RegOU	M	4	-17.4	44.7	7.7	0.001	3.0	0.790 \pm 0.584	0.193			
S+P	OLS	-	4	-17.5	45.0	6.7	-	3.5	0.859 \pm 0.534	0.123	0.135 \pm 0.254	0.602	
S	RegOU	C	4	-17.6	45.2	6.2	0.279	3.8	0.561 \pm 0.575	0.343			0.004 \pm 0.022
S+L	OLS	-	4	-17.7	45.3	5.8	-	4.0	0.897 \pm 0.531	0.106			0.004 \pm 0.022
S+F	RegPagel	M	5	-16.3	45.7	4.7	0.625	5.0	0.510 \pm 0.526	0.346	0.343 \pm 0.194	0.097	
S	RegPagel	C	4	-18.1	46.1	3.8	0.396	6.1	0.524 \pm 0.579	0.379			
S+F	RegOU	C	5	-16.6	46.3	3.5	0.442	6.7	0.249 \pm 0.579	0.673	0.324 \pm 0.197	0.119	
log Embryo dry weight													
S	OLS	-	3	-10.5	28.1	16.4	-	1.0	1.828 \pm 0.394				
S+F	OLS	-	4	-9.3	28.5	13.5	-	1.2	1.693 \pm 0.395		0.223 \pm 0.149	0.149	
S+L	OLS	-	4	-9.8	29.6	8.1	-	2.0	1.804 \pm 0.393				0.019 \pm 0.017
S	RegPagel	C	4	-9.9	29.8	7.2	0.434	2.3	1.939 \pm 0.417				
S	RegOU	M	4	-10.0	29.8	7.1	0.006	2.3	1.894 \pm 0.442	0.001			
S	RegPagel	M	4	-10.2	30.2	5.7	0.324	2.9	1.888 \pm 0.396				
S	RegOU	C	4	-10.4	30.6	4.7	0.315	3.5	1.853 \pm 0.436				
S+F	RegPagel	C	5	-8.9	30.9	4.2	0.333	3.9	1.804 \pm 0.421	0.001	0.173 \pm 0.142	0.240	
S+P	OLS	-	4	-10.5	30.9	4.0	-	4.1	1.822 \pm 0.408	0.000	0.016 \pm 0.194	0.934	
S+F	RegPagel	M	5	-9.0	31.0	3.9	0.162	4.2	1.758 \pm 0.389		0.193 \pm 0.151	0.217	
S+F	RegOU	M	5	-9.1	31.3	3.5	0.002	4.7	1.817 \pm 0.439		0.198 \pm 0.163	0.244	
S+L	RegPagel	C	5	-9.3	31.5	3.0	0.368	5.5	1.920 \pm 0.419				0.011 \pm 0.016
S+L	RegPagel	M	5	-9.3	31.6	2.9	0.284	5.7	1.872 \pm 0.392				0.020 \pm 0.018
S+F	RegOU	C	5	-9.3	31.6	2.9	0.177	5.7	1.735 \pm 0.427	0.001	0.193 \pm 0.152	0.224	
S+L	RegOU	M	5	-9.4	31.7	2.7	0.005	6.1	1.886 \pm 0.442	0.001			0.018 \pm 0.018
Allometric coefficient of ovary dry weight													
S+P	OLS	-	4	-21.1	52.1	45.5	-	1.0	2.955 \pm 0.612	<0.001	0.824 \pm 0.292	0.010	
S+P	RegPagel	M	5	-20.6	54.2	15.6	0.097	2.9	3.066 \pm 0.594	<0.001	0.796 \pm 0.294	0.015	
S+P	RegOU	C	5	-21.1	55.2	9.7	<0.001	4.7	2.955 \pm 0.612	<0.001	0.824 \pm 0.292	0.012	
S+P	RegOU	M	5	-21.1	55.2	9.7	<0.001	4.7	2.952 \pm 0.615	<0.001	0.824 \pm 0.292	0.012	

Model	Character Evolution	Tree k	lnML	AICc	w	λ or d	ER	Coefficients \pm SE (P-value)					
								log SL	Predation	Flow	Latitude		
log Ovary dry weight													
S	OLS	- 3	-11.0	29.2	25.4	-	1.0	2.739 \pm 0.402					
S	OLS	- 4	-9.9	29.8	19.0	-	1.3	2.652 \pm 0.398	<0.001	0.273 \pm 0.190	0.165		
S+L	OLS	- 4	-10.8	31.5	7.8	-	3.3	2.753 \pm 0.408			-0.011 0.017 0.527		
S+F	OLS	- 4	-11.0	31.9	6.6	-	3.9	2.708 \pm 0.421		0.051	0.159 0.751		
S	RegOU	C 4	-11.0	32.0	6.2	0.008	4.1	2.748 \pm 0.405					
S	RegOU	M 4	-11.0	32.0	6.2	<0.001	4.1	2.765 \pm 0.415					
S	RegPage1	M 4	-11.2	32.4	5.1	<0.001	5.0	2.769 \pm 0.387					
S	RegOU	C 5	-9.9	32.9	4.0	0.081	6.4	2.724 \pm 0.419	<0.001	0.283 \pm 0.190	0.157		
S	RegOU	M 5	-9.9	32.9	4.0	<0.001	6.4	2.715 \pm 0.436	<0.001	0.287 \pm 0.195	0.160		
S	RegPage1	M 5	-10.1	33.1	3.5	0.375	7.3	2.945 \pm 0.421	<0.001	0.330 \pm 0.197	0.113		
Matrotrophy Index													
S+L	OLS	- 4	12.1	-14.3	29.5	-	1.0	0.085 \pm 0.169	0.622		-0.014 \pm 0.007	0.069	
S	OLS	- 3	10.2	-13.2	17.5	-	1.7	0.067 \pm 0.178	0.710				
S	OLS	- 4	10.9	-11.9	9.1	-	3.2	0.035 \pm 0.179	0.846	0.100 \pm 0.085	0.255		
S+L	RegOU	C 5	12.1	-11.2	6.3	0.144	4.7	0.081 \pm 0.182	0.662			-0.014 \pm 0.008	0.091
S+L	RegOU	M 5	12.1	-11.2	6.2	0.001	4.8	0.160 \pm 0.193	0.418		0.041 \pm 0.070	0.560	
S+F	OLS	- 4	10.4	-10.8	5.2	-	5.7	0.042 \pm 0.185	0.823				
S	RegOU	C 4	10.2	-10.5	4.5	0.199	6.6	0.058 \pm 0.194	0.767				
S	RegOU	M 4	10.2	-10.5	4.5	0.003	6.6	0.177 \pm 0.203	0.396				
Principal Component 1													
P	RegOU	M 4	-49.1	108.2	30.6	0.015	1.0		1.428 \pm 0.867	0.118			
F	RegOU	M 4	-50.2	110.3	10.9	0.001	2.8			0.460 \pm 0.764	0.555		
F	OLS	- 3	-51.7	110.5	9.9	-	3.1			0.936 \pm 0.723	0.209		
L	RegOU	M 4	-50.3	110.6	9.2	0.005	3.3					-0.020 \pm 0.083	0.813
P	OLS	- 3	-51.8	110.7	8.6	-	3.6		1.094 \pm 0.919	0.247			
P	RegOU	C 4	-50.6	111.2	6.9	0.363	4.4		1.205 \pm 0.850	0.174			
F	RegOU	C 4	-51.1	112.2	4.2	0.245	7.3			0.633 \pm 0.727	0.397		
Principal Component 2													
L	OLS	- 3	-37.9	83.0	20.6	-	1.0					-0.073 \pm 0.048	0.140
F	OLS	- 3	-38.0	83.0	20.2	-	1.0					-0.648 \pm 0.427	0.143

Model	Character Evolution	Tree	k	lnML	AICc	w	λ or d	ER	Coefficients \pm SE (P-value)				
									log SL	Predation	Flow	Latitude	
P	OLS	-	3	-38.9	84.9	7.7	-	2.7		0.368 \pm 0.560	0.518		
F	RegPagel	C	4	-37.5	85.0	7.5	<0.001	2.8				-0.757 \pm 0.398	0.074
P+F+L	OLS	-	5	-36.3	85.6	5.4	-	3.8		0.175 \pm 0.576	0.764	-0.721 \pm 0.422	0.104
L	RegOU	C	4	-37.9	85.8	5.0	<0.001	4.1					-0.074 \pm 0.051
L	RegOU	M	4	-37.9	85.8	5.0	<0.001	4.1					-0.073 \pm 0.048
F	RegOU	C	4	-38.0	85.8	5.0	<0.001	4.1				-0.648 \pm 0.427	0.147
F	RegOU	M	4	-38.0	85.8	4.9	<0.001	4.2				-0.650 \pm 0.428	0.147
L	RegPagel	M	4	-38.3	86.5	3.6	<0.001	5.7					-0.081 \pm 0.051
													0.128

Appendix 2.3. List of software files used in analyses.

All files within the folder Gambusia/ANALYSES/LH/20120311

Input Data:

Life history data: Gambusia_LH_20110827.csv

Feeding habits of fish communities:

Gambusia_list_fish_community_20110811.csv

Site description: GAMBUSIA_MD_20120204.csv

Fish community in each site:

Gambusia_Predator_List_Per_Collection_20120516.csv

Folder with SI and related data txt files, used to corroborate SL:

Gambusia_SI_data

Code to process data:

Data input and preparation, matching databases; Table 1:

Gambusia_LH_Data_Preparation_20120516.R

Preparation of tree: Gambusia_LH_Tree_Preparation_20120313.R

Estimation of matrotrophy index, ranges; exporting data later used in

RegressionV2: Gambusia_LH_MIN_MAX_20120516.R

Folder with RegressionV2, files used to run the program, and folder with

RegressionV2 results: Phylogenetics_w_REGRESSIONv2

Processing of output from RegressionV2: Gambusia_LH_Comparative_After_
REGRESSIONv2_for_results_of_20120315c.R

Preparation of Figure 1: Gambusia_LH_Figure1_size_vs_traits_20120518.R

Preparation of Figure 2: Gambusia_LH_Figure 2_PCA_plot_20120517.R

Preparation of Figure 3: Gambusia_LH_Figure_SLvsGONADDRY_20120517.R

Chapter 3

Variation in body shape of Trinidadian guppies along a geomorphological gradient

Abstract: Disturbances are ubiquitous disruptive phenomena in nature, but organisms may respond adaptively to the selective forces that they exert. Here we study the body shape of guppies (*Poecilia reticulata*, Poeciliidae) in response to flow regime in mountain streams of Trinidad. In these habitats, floods are short-lived, severe, and may cause high mortalities of fish. We analyzed the body shape of females and males of 22 guppy populations from the Northern Range of Trinidad. We measured body shape with geometric morphometrics and with Streamlining Index. We tested the correlation between guppy shape and three geomorphological variables, watershed area, relief, and shape, which are indicative of flow regime. We also measured site characteristics that may influence fish shape, including pool volume, canopy cover, flow velocity, and the abundance of prawns and *Rivulus hartii* (previously known to ecologically interact with guppies). We observed a correlation between body shape and geomorphological features of the watersheds. However, the results varied between sexes. We predicted that guppies have larger caudal areas in more circular watersheds or in steeper watersheds, but we only observed the former in females and the latter in males. Despite our efforts to reduce environmental variation other than flow regime, we found gradients of prawn and *Rivulus*

abundances that explained some of the body shape variation. Surprisingly, watershed area was not a strong predictor of guppy shape. Additionally, we found that pregnancy dissipated the correlations between environmental variables and shape. We observed that body shape was partially explained by geographical proximity of the sites, specially the watershed of origin; however, the statistical significance of the results were almost the same when the hierarchical structure of the sampling was taken into account.

Introduction

Disturbances are ubiquitous phenomena (e.g. fires, hurricanes, droughts, floods) that disrupt and may even drive populations to extinction (Pickett and White 1985). However, organisms may have the potential to adapt to the strong selective forces that disturbances exert (Lytle 2001). Moreover, organisms adapted to certain patterns of disturbance may be negatively impacted by their alteration (for example by fire prevention, levees, dams, etc.; Lytle and Poff 2004). Whereas the ecological effects of disturbance have received considerable attention (Pickett and White 1985), their evolutionary consequences have been rarely addressed (Lytle 2001). Here we study the body shape of guppies in response to flow regime in mountain streams of Trinidad.

Floods in mountain streams are excellent phenomena to understand the ecological and evolutionary implications of disturbances. In such habitats, floods are short-lived but severe because adjacent steep banks confine waters to the stream channel (Swanson et al. 1998). Flash-floods are a recurrent disturbance that cause economic and social catastrophes (Wohl 2000), and are a major cause of mortality for aquatic fauna (Meffe 1984; Dawson 1988; Chapman and Kramer 1991; Peterson 1996; Lytle 2000; Fausch et al. 2001; Grether et al. 2001). For example, 13 populations of a molly in mountain streams of Costa Rica suffered an average of 75% reduction in size after a flash-flood (Chapman and Kramer 1991). Similarly, guppy populations living in six Trinidadian mountain

streams suffered 22-92% biomass reduction after a flooding event (Grether et al. 2001). Here we perform a comparative field study of guppy body shape across a gradient of flow regimes in rivers of the Northern Range of Trinidad.

Flow regime is more complex than just water flow velocity (Poff et al. 1997). Sites with the same base flow velocity can have very different patterns of flooding. These floods (and in general any disturbance) can be characterized by their frequency, magnitude, seasonality, and timing (Lytle and Poff 2004; Sabo and Post 2008). However, measuring these characteristics is very data-demanding (e.g., the daily flow time series should be at least 20 years long; Sabo and Post 2008), and such flow data are extremely rare for mountain streams (Wohl 2006). To overcome this issue, we relied on geomorphological variables known to be well correlated with flow regime: watershed area, relief, and shape (Gordon et al. 2004). Watershed area is a major factor in freshwaters, influencing stream flow, sediment transportation, streambed grain size, nutrient flux, and channel morphology (Strahler 1964; Church 2002). Larger watersheds collect more rainfall and consequently their floods discharge more water (i.e., flood disturbance have a higher frequency and magnitude). Watersheds with a steeper relief have stronger and shorter-lived floods because rainfall water runs down in a shorter time (i.e., a higher discharge peak; Gordon et al. 2004). Watershed shape also influences discharge peak. Whereas more rounded watersheds act as a funnel (i.e. rainfall water runs down in a short time period causing strong and short-lived flood), elongated watersheds work as a pipe (i.e., rainfall water is

evacuated with weaker and more prolonged flows; Strahler 1964). We predict that fish living in watersheds with larger areas, steeper relief, and more rounded shapes will have body shapes better suited for dealing with stronger floods. This morphology may be predicted by applying biomechanical theory to fish.

Biomechanical theory predicts that fish maximize swimming performance by having body shapes more efficient for the type of swimming they use. Streamlined shapes (outlines similar to foils that minimize flow drag) can cruise at constant velocity (steady swimming) for longer periods (Langerhans and Reznick 2010). Conversely, bodies with deeper caudal areas displace more water when undulating, generating more acceleration (unsteady swimming; Lighthill 1971; Webb 1982; Walker 1997). These two shapes thus exhibit a trade-off; maximizing unsteady swimming alters the outline in a way that increases drag (Langerhans 2009). We predict that fish from sites with stronger floods (i.e., with larger, steeper, and more rounded watersheds) will have deeper caudal areas. This morphology would allow them to reduce costly swimming in turbulent flows (Enders et al. 2003; Liao 2007), like those created by floods (Webb 2004), by optimizing unsteady (non-constant velocity) swimming (Langerhans and Reznick 2010). Since fish seem to show a morphological trade-off between the optimal streamlined shape for steady swimming and a larger caudal region optimal for unsteady swimming, we predict that fish from sites with stronger floods will depart more from the optimal streamlined shape.

We analyzed natural populations of Trinidadian guppies to test our hypotheses. The Trinidadian guppy system is well known for showing a gradient of predation pressure (Reznick and Endler 1982; Reznick et al. 1996b). Guppies from high-predation sites are exposed to piscivore fishes that readily prey on them, increasing their mortality rates (Reznick et al. 1996a). Multiple aspects of guppy phenotype are related with this predation gradient, including body shape (Hendry et al. 2006), coloration (Endler 1995), life history (Reznick and Endler 1982), and diet (Zandonà et al. 2011), among many others (Magurran 2005). Here we study the less-known geomorphological gradient of this system. Previous studies have hinted at the existence of this gradient. Reznick et al. (1996b; unpub. data) observed considerable variation in life histories of guppies even within low-predation sites in Trinidad. Similarly, Grether et al. (2001) observed large variation in the guppy biomass reduction after a flooding event that affected multiple low-predation Trinidadian sites. Here we focus on the effects this geomorphological gradient has on guppy morphology by minimizing variation due to predation regime (by selecting only low-predation sites). We also minimized the effect of flow velocity, a factor strongly correlated with body shape in fishes (Langerhans 2008), by exclusively sampling pools (i.e., sites with low flow velocity).

Methods

Source of specimens

Trinidadian guppies were collected from 22 wild populations (Table 3.1, Figure 3.1). Guppies are found in most Trinidadian streams in populations isolated from each other by waterfalls, land, and ocean. We focused on the Northern Range of Trinidad, a region with relatively homogeneous weather, soil, and vegetation cover (mostly primary and secondary forest; Cooper and Bacon 1981; Gopeesingh 1989; pers. obs.). Specifically, we sampled four watersheds, two on the North slope of the range mountains (Madamas and Paria), and two on the Southern slope of the range (Guanapo and Quare). We selected sites with contrasting geomorphological variation in watershed area, Relief Ratio, and Elongation Ratio (see Habitat Characterization below). We also applied previous knowledge of the fish communities of these watersheds, and selected only those reaches that were categorized as low predation (LP) sites. These LP sites lack piscivorous fish (e.g., *Hoplias malabaricus* and *Crenicichla alta* in the South slope, *Eleotris pisonis*, *Gobiomorus dormitor*, and *Dorritator maculatus* in the North slope; Reznick et al., 1996). However, LP sites have other aquatic organisms that can exert relatively mild predation pressure on guppies. All LP sites have the ubiquitous *Rivulus hartii*, an omnivore that rarely preys on guppies (Magurran 2005). LP sites in the Guanapo and Madamas have *Rhamdia quelen*, an omnivore that feeds mainly on invertebrates and plant material, and rarely fish

(Maldonado-Ocampo et al. 2006). In the Quare, Paria, and Madamas, there are prawns (*Macrobrachium* spp.), which may feed on guppies (Magurran 2005; pers. obs.). North-slope sites (Paria and Madamas) have *Agonostomus monticola* and *Sicydium punctatum*. The former species mainly feeds on invertebrates, plant material and algae (Phillip 1993), and the latter on algae (Coat et al. 2009).

In each site we looked for a population of guppies living in a pool delimited up- and downstream by waterfalls. Fish were captured with hand nets, an attempt was made to include the full range of body sizes for each sex. Soon after capture, fish were euthanized in MS-222 and preserved in 5% formalin. For sites that were sampled more than once, we pooled together individuals after confirming that the distribution of their shape variables was not statistically different. After excluding juveniles and bent specimens, we analyzed 568 females and 386 males (Table 3.1).

Habitat characterization

Three characteristics of the watershed were measured from cartographic maps (1:25000 scale), using Adobe Photoshop CS5 (Adobe Systems, Seattle, WA, USA). Watershed area (AREA) was the projected drainage area upstream of each site. The steepness of the watershed was measured with the Relief Ratio (REL), the elevation difference between site and maximum watershed height, divided by maximum watershed length (Schumm 1956). Steeper watersheds

have larger REL values. The shape of the watershed was quantified with the Elongation Ratio (ELO), the ratio between the diameter of a circle with the same area of the watershed, and the maximum watershed length (Morisawa 1958). ELO values range from less than one, indicating elongated watersheds, to values close to one for more circular watersheds.

We aimed to minimize environmental variation not related with the flow regime by sampling only pools from LP localities with pristine basins. Nevertheless, we measured five local variables known to influence guppy phenotypes to test whether shape variation was better explained by factors different from the three geomorphological watershed variables. Pool volume (VOL) was calculated as one half of an ellipsoid ($4\pi abc/3$, where a =maximum pool width, b = maximum pool length, and c =maximum pool depth). Lengths were measured with a laser distance measurer (Leica Disto D5), and depths with a measuring rod coupled with a rod level. The percentage of canopy cover (CAN) was measured with a concave spherical densiometer (Forest Densiometers, Bartlesville, OK), averaging measurements made in all four cardinal directions (from the same point). Water flow velocity (VEL) was measured with an electromagnetic flow meter (Marsh-McBirney Flo-Mate Model 2000) at the deepest point of the pool, positioning the probe at a depth equal to maximum depth (Gordon et al. 2004). The abundance of prawn (PRW) and *R. hartii* (RIV) was estimated using a catch per unit effort (CPUE) measure. Two collapsible minnow traps (Models TR-501 and TR-503, Promar Company, Gardena, CA)

were baited with five dog food pellets and left undisturbed for 15 minutes. The traps were placed in portions of the pool where guppies were observed and water depth was deep enough to cover the entrances of the traps. The relative abundance of prawns and *R. hartii* was the number of individuals of each taxa collected in both traps.

Geographic coordinates were measured in each site using Global Positioning System equipment, which was set to the Universal Transverse Mercator projection and the Naparima 1955 datum.

Photographs and Dissections

Lateral and ventral views of preserved fish were photographed following the recommendations of Zelditch et al. (2004). Standard length (SL) of specimens was measured with calipers (to the nearest 0.1 mm). Females were dissected to estimate stage of embryo development using the scale of Haynes (1995), as modified by Pires et al. (2007). Stages in this scale range from egg (stage 0) to fully-formed pre-birth embryo (stage 50). We only included mature individuals to eliminate the morphological variation associated with immaturity. For females, we excluded individuals smaller than the minimum size at maturity for each collection (i.e., the SL of the smallest female with stage of development of embryos ≥ 2). For males, individuals without a fully formed gonopodial tip were excluded.

Measurements of shape

Shape was measured with two complimentary methods. The first method was two-dimensional geometric morphometrics (Zelditch et al. 2004). Eleven landmarks (homologous coordinates) and five sliding landmarks (non-homologous coordinates along the outline) were digitized on lateral photographs using TpsDig2 2.12 (Rohlf 2008), as explained in Appendix 3.1. Semilandmarks were slid to minimize the bending energy criterion (Zelditch et al. 2004), with code adapted from Morpho (Stefan Schlager, unpub). Landmarks and slid semilandmarks were Procrustes superimposed (to extract variation independent of location, scale, and rotation of the specimens in the images) using R-code adapted from Claude (2008).

Three mean-shape configurations were estimated for guppies from each site: mean-size female early in pregnancy (when embryos were at developmental stage 0), mean-size female late in pregnancy (embryos at stage 50), and mean-size male. Body size was measured as the centroid size (CS; Zelditch et al. 2004). For females, the two morphologies were estimated using a multiple regression model (Monteiro 1999; Berner 2011), with projected landmark positions as dependent variables, and centroid size and stage of development of embryos as independent variables. Dimensionality was reduced with a Principal Component Analysis on the superimposed coordinates for each of the three configuration sets, retaining 95% of the variation. Principal Component scores

were used in further analyses. Modified thin-plate splines were used to visualize shape differences, as explained in Chapter 1.

The second method used to estimate shape was the tridimensional estimation of body mass distribution of the Streamlining Index (McHenry and Lauder 2006). SI is a ratio of the mass distributions of a fish over an optimally streamlined foil of the same length, width, and depth as the fish (McHenry and Lauder 2006). SI ranges from less than one (proportionally larger anterior body regions), to one (optimal body mass distribution along the longitudinal fish axis), to more than one (proportionally larger caudal regions). SI was calculated using Matlab 7.5 with Image Processing Toolbox 6.0 (MathWorks, Natick, MA), as explained in Chapter 1. As in the geometric morphometric analyses, three SI values were estimated for the hypothetical averaged-size individual of each sex in each population: one value for females early in pregnancy, another for late-in-pregnancy females, and the last for males.

Statistical analyses

Thirteen adaptive hypotheses were tested for each dependent variable (DV):

$$DV \sim CS + \text{Watershed} + \varepsilon$$

$$DV \sim CS + \text{AREA} + \text{Watershed} + \varepsilon$$

$$DV \sim CS + \text{REL} + \text{Watershed} + \varepsilon$$

$$DV \sim CS + \text{ELO} + \text{Watershed} + \varepsilon$$

$$DV \sim CS + \text{CAN} + \text{Watershed} + \varepsilon$$

$$DV \sim CS + VOL + Watershed + \varepsilon$$

$$DV \sim CS + VEL + Watershed + \varepsilon$$

$$DV \sim CS + PRAWN + Watershed + \varepsilon$$

$$DV \sim CS + RIV + Watershed + \varepsilon$$

$$DV \sim CS + AREA + REL + ELO + Watershed + \varepsilon$$

$$DV \sim CS + CAN + VOL + VEL + Watershed + \varepsilon$$

$$DV \sim CS + PRW + RIV + Watershed + \varepsilon$$

$$DV \sim CS + AREA + REL + ELO + CAN + VOL + VEL + PRW + RIV + Watershed + \varepsilon,$$

where AREA, CAN, VOL, VEL, PRW, and RIV were log-transformed values, ε was the error term, and DV were the geometric morphometric shape variables, CS, or SI (more details below). These models were evaluated with Ordinary Least Squares (OLS) and with a phylogenetic framework to account for the potentially hierarchical structure of the data, which could be produced by the hierarchical nature of river networks. The phylogeny used originally had branch lengths proportional to geographical distances between sites along the streambed (Appendix 3.2). To prevent the negative effect of some disproportionately long branches on the comparative analyses, branch lengths were transformed using Grafen's (1989) power transformation (Appendix 3.3). One soft polytomy at the base of the river watershed tree was resolved prior to analyses by making the tree dichotomous (function multi2di in the package ape; Paradis et al. 2004) and setting the branch length of the new branches to zero

length. Type I error produced by this polytomy was corrected by reducing the degrees of freedom when estimating P-values (Garland and Díaz-Uriarte 1999).

Two different methods were used to test the adaptive hypotheses, depending on whether the dependent variables were geometric morphometric shape variables or not (CS or SI). For the former, we used phylogenetic MANCOVAs (Garland et al. 1993; Revell et al. 2007; Chapter 1), with PC scores as dependent variables, watershed as a factor, and other predictors (CS, AREA, REL, ELO, VOL, CAN, VEL, PRAWN, and RIV) as covariates. We tested two models of character evolution, OLS (non-phylogenetic) and Brownian Motion (BM, random divergence). For the phylogenetic MANCOVA, the P-values of the F-statistics obtained from OLS were estimated using an empirical null distribution calculated from Monte Carlo simulations of tip data (999 simulations), using the original topology as the phylogenetic structure, and observed root values as starting values. To visualize the shape variation explained by predictors of MANCOVA, we used a canonical analysis (Langerhans 2009; Chapter 1). We calculated the phylogenetic signal (K; Blomberg et al. 2003) and the probability of K=0 for each canonical vector, using *phylosignal* in the R package *picante* (Kembel et al. 2010).

When the dependent variables were CS or SI, we used regression models to analyze the adaptive hypotheses. Specifically, we used phylogenetic Generalized Least Squares (GLS; Grafen 1989), which has the option of hierarchically structuring the error term by multiplying it by a phylogenetic

variance-covariance matrix (Martins and Hansen 1997; Garland and Ives 2000; Rezende and Diniz-Filho 2012). We tested three models of character evolution, OLS (non-phylogenetic, the error term multiplied by the identity matrix), Pagel's (RegPagel, a regression model that assumes Brownian motion and simultaneously estimates the parameter coefficients and a measurement of phylogenetic signal; Pagel 1999; Garland et al. 2005), and the Ornstein–Uhlenbeck process (RegOU, which assumes Brownian motion that tends to drift towards an optimum determined by selection and simultaneously estimates regression coefficients and a measure of phylogenetic signal; Hansen 1997; Butler and King 2004). When CS was the dependent variable, it was not used as a covariate in the models.

For both MANCOVA and GLS analyses, we selected the best supported model for each dependent-variable set using model selection (Burnham and Anderson 2002). Because of our relatively small sample size, we used small sample corrected Akaike information criterion (AICc; Anderson and Burnham 2002; Hansen et al. 2008). Akaike weights (w , the amount of evidence explained by each model) and Evidence Ratios (ER, the number of times each model is worse than the best supported model) were also estimated. GLS regressions were calculated using the Matlab program RegressionV2.m (A. R. Ives and T. Garland Jr.; Lavin et al. 2008). Other analyses were executed using code written in R (R Development Core Team 2011).

Results

Some of the habitat characteristics (Table 3.1) were strongly correlated (Table 3.2). Specifically, larger watershed area was associated with larger pool volume and larger water flow velocity; steeper relief was associated with more elongated watersheds and a reduction in canopy cover.

MANCOVAS of geometric morphometric shape variables

In females, the best supported MANCOVAs (for both sets of configurations, early and late in pregnancy) were the adaptive models with all terms ($\Delta AIC_C > 8$). For females early in pregnancy, AREA was the only geomorphological variable significantly related with geometric morphometric shape variables, other significantly related predictors were biotic (CS, PRW, and RIV; Table 3.3, Figure 3.2). The divergence (d) vectors of these significant results showed phylogenetic signal (Table 3.3). The Phylogenetic MANCOVA showed that only AREA, PRW, and RIV were significant after accounting for phylogenetic relatedness (Table 3.3). The d vectors for AREA and PRW (but not RIV), were significantly related with SI (Table 3.3), indicating that early-in-pregnancy females have proportionally larger anterior areas in sites with larger watershed areas and more prawns (Figure 3.2). Female shape late in pregnancy showed the same tendencies as early in pregnancy, but none of the p-values were significant (results not shown).

In males, the best supported MANCOVA was the full model (ΔAIC_C between best and second best supported models was 20.488). For males, only REL and PRW were significantly predictors of shape, in both OLS and phylogenetic MANCOVAs (Table 3.4). The divergence vectors of these two relationships showed significant levels of phylogenetic signal (Table 3.4). Both vectors were positively related with SI, indicating that species living in steeper watersheds and in sites with more prawns have larger caudal areas (Table 3.4, Figure 3.2).

Regressions of CS and SI

Centroid size of females and males were best explained by watershed; specimens from the Guanapo river were larger (Figure 3.3 A-B). For females, CS was also related with VOL; larger females were found in larger pools (Figure 3.3 A). The best supported model for SI of early in pregnancy females was $SI \sim CS + ELO + watershed$ and explained 36.7% of the variation (w value, Appendix 3.4). In this model, SI early in pregnancy was independent of CS ($p=0.420$), but was correlated with ELO (estimated coef. $\pm SE = 0.346 \pm 0.133$, $p = 0.021$) and watershed ($p=0.021$). The best supported model for SI of females late in pregnancy was $SI \sim CS + REL + watershed$, but none of the predictors in this or in the other models was statistically significant. For males, SI was best explained by the model $SI \sim CS + VEL + watershed$ ($w=60.7$, Appendix 3.4). In this model, CS

was not correlated with SI ($p=0.949$), but VEL (0.200 ± 0.073 , $p=0.016$) and watershed ($p= 0.002$) were.

Discussion

Here we tested the correlation between guppy shape and three geomorphological variables indicative of flow regime. We observed a correlation between body shape and geomorphological features of the watersheds. However, the results varied between sexes. We predicted that guppies have larger caudal areas in more circular (funnel-shape) watersheds or in steeper watersheds, but we only observed the former in females (Figure 3.3) and the latter in males (Figure 3.2). Despite our efforts to reduce environmental variation other than flow regime, we found gradients of prawn and *Rivulus* abundances that explained some of the body shape variation (Tables 3.3-3.4, Figure 3.2). Surprisingly, watershed area was not a strong predictor of guppy shape (Figures 3.2-3.3). Additionally, we found that pregnancy dissipated the correlations between environmental variables and shape. We observed that body shape was partially explained by geographical proximity of the sites, specially the watershed of origin; however, the statistical significance of the results were almost the same when the hierarchical structure of the sampling was taken into account.

Some relationships between flow regime (estimated by geomorphological features) and body shape matched our predictions. There are several examples

of flow regime effects on freshwater fish. The structure of fish communities is so well correlated with aspects of flow regime that it can be used as their indirect index (Chang et al. 2011; Huang et al. 2011). Flow regime not only affects the structure of the fish community, but also the life history traits of the species in the community (Mims et al. 2010). Flow regime variation also affects fish at the intraspecific level. In *Cyprinella robusta*, a small cyprinid, populations experiencing human-made alteration of the flow regime (river impoundment) showed a small but consistent change in body shape, specially in the size and orientation of the head (Haas et al. 2010). However, to the best of our knowledge, the present study is the first one to analyze the correlation between a natural geomorphological gradient and fish body shape.

The association between geomorphology and body shape morphology was not consistent between sexes. Guppy body shape is sexually dimorphic, females have larger abdomens and males have more anterior anal fins (Hendry et al. 2006; Figure 3.2). According to our results, female and male guppies may be responding differently to selection pressures that flow regime may be causing. These differences between sexes are likely independent of pregnancy. We accounted for pregnancy in this study, observing that pregnancy reduced the phenotypic divergence across environments, what coincides with previous studies that reported pregnancy as a burden that reduces adaptive divergence (Ghalambor et al. 2004; Wesner et al. 2011; Chapter 1). Instead of a pregnancy effect, the intersexual differences observed here may lay on intersexual

differences in mortality regimes. This idea was previously suggested in a study that found intersexual differences in guppy body shape across gradients of predation, water flow velocity, and canopy cover (Hendry et al. 2006). This hypothesis is supported by the observation that males suffer higher mortality rates in high-predation sites than females (Reznick et al. 1996a). In the case of the mortality exerted by flow regime, there is evidence that males are recaptured less frequently than females after mountain flooding episodes (Chapman and Kramer 1991). The intersexual differences in shape and size in this species may be associated with differences in swimming performance, which in turn may confer fitness advantages when facing selective events occurring at peak flows.

We also found inconsistencies in the results obtained with different variables used to measure shape (geometric morphometrics and Streamlining Index). In females, geometric morphometric shape variables were best explained by AREA, PRW, and RIV (Table 3.3), whereas SI was more strongly correlated with ELO. Similarly, in males the shape variation depicted by geometric morphometrics was correlated with REL and PRW, whereas male SI was correlated with VEL. The discrepancy between the results using different methods is not surprising, geometric morphometrics is a very good method to condense shape variation (Zelditch et al. 2004), but the variable extracted may not necessarily be related with biomechanical function (Chapter 1). The results using these two commonly used methods should be seen as complementary rather than fully overlapping (Chapter 1).

Watershed relief, shape, and (indirectly) area, were related with fish shape in a pattern that matched our predictions. Larger caudal areas were observed in males from steeper relief watersheds and in females from more rounded watersheds. Regarding watershed area, females early in pregnancy had slightly deeper bodies in sites with larger basins (Figure 3.2). Since watershed area and flow velocity were strongly correlated (Table 3.2), the result of males having larger caudal areas in sites with stronger water flow velocity could be also interpreted as a watershed area effect. Despite all these significant results, given the large influence of watershed area in multiple stream ecology factors (Church 2002), we were expecting a larger watershed area effect. Our use of watershed area as a proxy to estimate flow regime characteristics has been done before (Lytle et al. 2008). Our sampling sites did not lack variation in watershed area, they ranged from 0.06 to 20.50 km². Instead, it may be that watershed area is a complex factor that correlates with multiple habitat characteristics, which ultimately may balance-out each other effects on the flow regime. For example, low stream order streams have smaller areas, what reduces flow peak height, but typically have steep slope and a reduced floodplain, what increases flow peak height (Church 2002). Recent analysis of the very few available hydrological datasets with high geographical density for mountain streams (e.g., Pike and Scatena 2010) are a promising strategy to understand the fine scale relationships between geomorphology of mountain streams and flow regime.

We found a relationship between body shape (as measured with geometric morphometrics) and the abundance of prawns in both sexes. We originally measured this variable to confirm that it had no effect on guppy body shape in our homogeneous site sample (as occurred for canopy cover). We found that both sexes coincided in having proportionally larger areas in sites with more prawns (Tables 3.3-3.4 and Figure 3.2). It could be hypothesized that this is a watershed effect, prawn populations went extinct in the Guanapo river few decades ago (D. Reznick, pers. obs.), they are rare in the Quare river, and are common in Northern range streams. Our analysis, however, accounted for watershed effects in the models, and yet prawn abundance showed a significant relationship with guppy shape. The observed relationship was actually counterintuitive, fish from sites with more prawns had larger abdominal areas, but if prawns are predators of fish and escaping from prawns utilizes unsteady swimming, guppies with higher prawn density should have had larger caudal areas. An alternative explanation could be that instead of predators, prawns act as competitors of guppies. Prawns have an omnivorous diet mainly based in detritus, algae, and invertebrates, and only in minor proportions containing fish (Lewis et al. 1966; Collins and Paggi 1997; Albertoni et al. 2003). This diet largely overlaps with guppy feeding habits (Zandonà et al. 2011). If prawns and guppies compete for food, guppies in sites with more prawns would have to search more for food, what would require a more frequent use of steady swimming, and

therefore more streamlined bodies (Langerhans and Reznick 2010) with less prominent caudal areas.

We also found that females early in pregnancy have shallower bodies in sites where *Rivulus* are more abundant (Table 3.3, Figure 3.2). This variation was not significantly related with SI (Table 3.3), therefore it is not indicative of differences in swimming performance. The interaction between guppies and *Rivulus* is complex, each species seems to prey on each other juveniles (Magurran 2005), and adults compete for food resources (de Villemereuil and López-Sepulcre 2011). The functional explanation of the shape response to *Rivulus* abundance observed here is not clear.

There are some future directions that could complement the results of this study. One limitation of this study was the use of wild populations, which may be affected by unmeasured environmental aspects. This caveat of any comparative study (Garland and Adolph 1991; Garland et al. 2005) could be overcome by a "common garden" experiment that keep populations in a controlled environment for one or more generations. Using a common garden approach it would be possible to estimate the proportion of phenotypic divergence that corresponds to phenotypic plasticity and to genetic variation. Nevertheless, despite we ignore whether the shape variation observed here has a plastic or genetic origin, our observations are indicative of morphological adaptive divergence.

Another important aspect not explored here is the behavioral response of fish to flow regime. Fish escape from flushing flows by orienting their body with

the direction of flow as soon as water velocity increases (Meffe 1984), by hiding behind barriers (Meffe 1984; Liao 2007), or by swimming close to the shore where stream flow velocity is reduced (pers. obs.). A more complete ecomorphological picture (Garland and Losos 1994) of the effect of flow regime on fish should include morphology, behavior, and fitness measures.

Human impact is dramatically changing flow regimes all around the world, specially in the delicate mountain rivers (Wohl 2006). Mountain rivers are important ecosystems for biodiversity and human development, but are suffering from an increasing anthropogenic pressure (e.g. by damming, water extraction for irrigation, channelization, and deforestation). Moreover, ongoing climate change is expected to increase the frequency and magnitude of flash-floods (Walters and Post 2008), making urgent a better understanding of the biological implications of such disturbances. Our study goes in that direction, we showed here that guppy body shape responds adaptively to flow regime.

Literature Cited

- Albertoni, E. F., C. Palma-Silva, and F. d. A. Esteves. 2003. Natural diet of three species of shrimp in a tropical coastal lagoon. *Brazilian Archives of Biology and Technology* 46:395-403.
- Anderson, D. R., and K. P. Burnham. 2002. Avoiding pitfalls when using information-theoretic methods. *The Journal of Wildlife Management* 66:912-918.
- Berner, D. 2011. Size correction in biology: how reliable are approaches based on (common) principal component analysis? *Oecologia* 166:961-971.
- Blomberg, S. P., T. Garland, Jr, and A. R. Ives. 2003. Testing for phylogenetic signal in comparative data: behavioral traits are more labile. *Evolution* 57:717-745.
- Burnham, K. P., and D. R. Anderson. 2002. Model selection and multimodel inference: a practical information-theoretic approach. Springer Verlag, New York, NY.
- Butler, M. A., and A. A. King. 2004. Phylogenetic comparative analysis: a modeling approach for adaptive evolution. *Am. Nat.* 164:683-695.
- Chang, F.-J., W.-P. Tsai, T.-C. Wu, H.-k. Chen, and E. E. Herricks. 2011. Identifying natural flow regimes using fish communities. *Journal of Hydrology* 409:328-336.

- Chapman, L. J., and D. L. Kramer. 1991. The consequences of flooding for the dispersal and fate of poeciliid fish in an intermittent tropical stream. *Oecologia* 87:299-306.
- Church, M. 2002. Geomorphic thresholds in riverine landscapes. *Freshwat. Biol.* 47:541-557.
- Claude, J. 2008. *Morphometrics with R*. Springer, New York.
- Coat, S., D. Monti, C. Bouchon, and G. Lepoint. 2009. Trophic relationships in a tropical stream food web assessed by stable isotope analysis. *Freshwat. Biol.* 54:1028-1041.
- Collins, P., and J. Paggi. 1997. Feeding ecology of *Macrobrachium borelli* (Nobili) (Decapoda: Palaemonidae) in the flood valley of the River Paraná, Argentina. *Hydrobiologia* 362:21-30.
- Cooper, G. C., and P. R. Bacon, eds. 1981. *The natural resources of Trinidad and Tobago*. Edward Arnold Ltd, London, UK.
- Dawson, F. S. 1988. Water flow and the vegetation of running waters. Pp. 283-309 in J. J. Symoens, ed. *Vegetation of inland waters*. Kluwer, London.
- de Villemereuil, P. B., and A. López-Sepulcre. 2011. Consumer functional responses under intra- and inter-specific interference competition. *Ecol. Model.* 222:419-426.

- Enders, E. C., D. Boisclair, and A. G. Roy. 2003. The effect of turbulence on the cost of swimming for juvenile Atlantic salmon (*Salmo salar*). *Can. J. Fish. Aquat. Sci.* 60:1149-1160.
- Endler, J. A. 1995. Multiple-trait coevolution and environmental gradients in guppies. *Trends in Ecology & Evolution* 10:22-29.
- Fausch, K. D., Y. Taniguchi, S. Nakano, G. D. Grossman, and C. R. Townsend. 2001. Flood disturbance regimes influence rainbow trout invasion success among five Holarctic regions. *Ecol. Appl.* 11:1438-1455.
- Garland, T., Jr, and S. C. Adolph. 1991. Physiological differentiation of vertebrate populations. *Annu. Rev. Ecol. Syst.* 22:193-228.
- Garland, T., Jr, A. F. Bennett, and E. L. Rezende. 2005. Phylogenetic approaches in comparative physiology. *J. Exp. Biol.* 208:3015-3035.
- Garland, T., Jr, and R. Díaz-Uriarte. 1999. Polytomies and phylogenetically Independent Contrasts: examination of the bounded degrees of freedom approach. *Syst. Biol.* 48:547-558.
- Garland, T., Jr, A. W. Dickerman, C. M. Janis, and J. A. Jones. 1993. Phylogenetic analysis of covariance by computer simulation. *Syst. Biol.* 42:265-292.
- Garland, T., Jr., and A. R. Ives. 2000. Using the past to predict the present: confidence intervals for regression equations in phylogenetic comparative methods. *The American Naturalist* 155:346-364.

- Garland, T., Jr., and J. B. Losos. 1994. Ecological morphology of locomotor performance in squamate reptiles. Pp. 240-302 in P. C. Wainwright, and S. M. Reilly, eds. *Ecological Morphology: Integrative Organismal Biology*. Chicago University Press, Chicago.
- 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*). *The American Naturalist* 164:38-50.
- Gopeesingh, S. 1989. Hydrogeological map of Trinidad. Water and Sewerage Authority, Government of Trinidad and Tobago, Port of Spain, Trinidad and Tobago.
- Gordon, N. D., T. A. McMahon, B. L. Finlayson, C. J. Gippel, and R. J. Nathan. 2004. *Stream Hydrology: an introduction for ecologists*. John Wiley & Sons, West Sussex, UK.
- Grafen, A. 1989. The Phylogenetic Regression. *Philosophical Transactions of the Royal Society of London. Series B, Biological Sciences* 326:119-157.
- Grether, G. F., D. F. Millie, M. J. Bryant, D. N. Reznick, and W. Mayea. 2001. Rain forest canopy cover, resource availability, and life history evolution in guppies. *Ecology* 82:1546-1559.
- Haas, T. C., M. J. Blum, and D. C. Heins. 2010. Morphological responses of a stream fish to water impoundment. *Biol. Lett.* 6:803-806.

- Hansen, T. F. 1997. Stabilizing selection and the comparative analysis of adaptation. *Evolution* 51:1341-1351.
- Hansen, T. F., J. Pienaar, and S. H. Orzack. 2008. A comparative method for studying adaptation to a randomly evolving environment. *Evolution* 62:1965-1977.
- Haynes, J. L. 1995. Standardized classification of Poeciliid development for life-history studies. *Copeia* 1995:147-154.
- 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. *J. Evol. Biol.* 19:741-754.
- Huang, F., Z. Xia, N. Zhang, and Z. Lu. 2011. Does hydrologic regime affect fish diversity? -A case study of the Yangtze Basin (China). *Environ. Biol. Fishes* 92:569-584.
- Kembel, S. W., P. D. Cowan, M. R. Helmus, W. K. Cornwell, H. Morlon, D. D. Ackerly, S. P. Blomberg, and C. O. Webb. 2010. Picante: R tools for integrating phylogenies and ecology. *Bioinformatics* 26:1463-1464.
- Langerhans, R. B. 2008. Predictability of phenotypic differentiation across flow regimes in fishes. *Integr. Comp. Biol.* 48:750-768.
- Langerhans, R. B. 2009. Trade-off between steady and unsteady swimming underlies predator-driven divergence in *Gambusia affinis*. *J. Evol. Biol.* 22:1057-1075.

- Langerhans, R. B., and D. N. Reznick. 2010. Ecology and evolution of swimming performance in fishes: predicting evolution with biomechanics. Pp. 200-248 in P. Domenici, and B. G. Kapoor, eds. Fish Locomotion: an etho-ecological perspective. Science Publishers, Enfield.
- Lavin, S. R., W. H. Karasov, A. R. Ives, K. M. Middleton, and T. Garland Jr. 2008. Morphometrics of the avian small intestine compared with that of nonflying mammals: a phylogenetic approach. *Physiol. Biochem. Zool.* 81:526-550.
- Lewis, J. B., J. Ward, and A. McIver. 1966. The breeding cycle, growth and food of the fresh water shrimp *Macrobrachium carcinus* (Linnaeus). *Crustaceana* 10:48-52.
- Liao, J. C. 2007. A review of fish swimming mechanics and behaviour in altered flows. *Philosophical Transactions of the Royal Society B: Biological Sciences* 362:1973-1993.
- Lighthill, M. J. 1971. Large-amplitude elongated-body theory of fish locomotion. *Proceedings of the Royal Society of London. Series B. Biological Sciences* 179:125-138.
- Lytle, D. A. 2000. Biotic and abiotic effects of flash flooding in a montane desert stream. *Arch. Hydrobiol.* 150:85-100.
- Lytle, D. A. 2001. Disturbance regimes and life-history evolution. *The American Naturalist* 157:525-536.

- Lytle, D. A., M. T. Bogan, and D. S. Finn. 2008. Evolution of aquatic insect behaviours across a gradient of disturbance predictability. *Proceedings of the Royal Society B: Biological Sciences* 275:453-462.
- Lytle, D. A., and N. L. Poff. 2004. Adaptation to natural flow regimes. *Trends Ecol. Evol.* 19:94-100.
- Magurran, A. E. 2005. *Evolutionary Ecology: the Trinidadian guppy*. Oxford University Press, Oxford, UK.
- Maldonado-Ocampo, J., A. Ortega-Lara, J. S. Usma, F. A. Villa-Navarro, L. Vásquez, S. Prada-Pedrerros, and C. Ardila. 2006. *Peces de los Andes de Colombia*. Instituto de Investigación de Recursos Biológicos Alexander von Humboldt, Bogotá D.C.
- Martins, E. P., and T. F. Hansen. 1997. Phylogenies and the comparative method: A general approach to incorporating phylogenetic information into the analysis of interspecific data. *Am. Nat.* 149:646-667.
- McHenry, M. J., and G. V. Lauder. 2006. Ontogeny of form and function: Locomotor morphology and drag in zebrafish (*Danio rerio*). *J. Morphol.* 267:1099-1109.
- Meffe, G. K. 1984. Effects of abiotic disturbance on coexistence of predator-prey fish species. *Ecology* 65:1525-1534.
- Mims, M. C., J. D. Olden, Z. R. Shattuck, and N. L. Poff. 2010. Life history trait diversity of native freshwater fishes in North America. *Ecol. Freshwat. Fish* 19:390-400.

- Monteiro, L. R. 1999. Multivariate Regression Models and Geometric Morphometrics: The Search for Causal Factors in the Analysis of Shape. *Syst. Biol.* 48:192-199.
- Morisawa, M. 1958. Measurement of drainage-basin outline form. *The Journal of Geology* 66:587-591.
- Pagel, M. 1999. Inferring the historical patterns of biological evolution. *Nature* 401:877-884.
- Paradis, E., J. Claude, and K. Strimmer. 2004. APE: Analyses of Phylogenetics and Evolution in R language. *Bioinformatics* 20:289-290.
- Peterson, C. G. 1996. Response of benthic algal communities to natural physical disturbance. Pp. 375-402 *in* R. J. Stevenson, M. L. Bothwell, and R. L. Lowe, eds. *Algal Ecology*. Academic Press, San Diego, CA.
- Phillip, D. 1993. Reproduction and feeding of the mountain mullet, *Agonostomus monticola*, in Trinidad, West Indies. *Environ. Biol. Fishes* 37:47-55.
- Pickett, S. T. A., and P. S. White. 1985. The ecology of natural disturbance and patch dynamics. Academic Press, San Diego, CA.
- Pike, A. S., and F. N. Scatena. 2010. Riparian indicators of flow frequency in a tropical montane stream network. *Journal of Hydrology* 382:72-87.
- 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 Part A: Ecological Genetics and Physiology* 307A:113-125.

- Poff, N. L., J. D. Allan, M. B. Bain, J. R. Karr, K. L. Prestegard, B. D. Richter, R. E. Sparks, and J. C. Stromberg. 1997. The natural flow regime. *Bioscience* 47:769-784.
- R Development Core Team. 2011. R: A language and environment for statistical computing. R Foundation for Statistical Computing, Vienna.
- Revell, L. J., L. J. Harmon, R. B. Langerhans, and J. J. Kolbe. 2007. A phylogenetic approach to determining the importance of constraint on phenotypic evolution in the neotropical lizard *Anolis cristatellus*. *Evol. Ecol. Res.* 9:261-282.
- Rezende, E. L., and J. A. F. Diniz-Filho. 2012. Phylogenetic analyses: comparing species to infer adaptations and physiological mechanisms. *Comprehensive Physiology* 2:639-674.
- 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. J. B. Iv, F. H. Rodd, and P. Ross. 1996a. Life-history evolution in guppies (*Poecilia reticulata*) 6. Differential mortality as a mechanism for natural selection. *Evolution* 50:1651-1660.
- Reznick, D. N., F. H. Rodd, and M. Cardenas. 1996b. Life-history evolution in guppies (*Poecilia reticulata*: Poeciliidae). IV. Parallelism in life-history phenotypes. *The American Naturalist* 147:319-338.

- Rohlf, F. J. 2008. tpsDig2. Distributed by the author. Department of Ecology and Evolution, State University of New York, Stony Brook.
- Sabo, J. L., and D. M. Post. 2008. Quantifying periodic, stochastic, and catastrophic environmental variation. *Ecol. Monogr.* 78:19-40.
- Schumm, S. A. 1956. Evolution of drainage systems and slopes in badlands at Perth Amboy, New Jersey. *Geological Society of America Bulletin* 67:597-646.
- Strahler, A. N. 1964. Quantitative geomorphology of drainage basins and channel networks. Pp. 39-73 *in* V. t. Chow, ed. *Handbook of applied hydrology*. McGraw Hill, New York.
- Swanson, F. J., S. L. Johnson, S. V. Gregory, and S. A. Acker. 1998. Flood disturbance in a forested mountain landscape. *Bioscience* 48:681-689.
- Walker, J. A. 1997. Ecological morphology of lacustrine threespine stickleback *Gasterosteus aculeatus* L. (*Gasterosteidae*) body shape. *Biol. J. Linn. Soc.* 61:3-50.
- Walters, A. W., and D. M. Post. 2008. An experimental disturbance alters fish size structure but not food chain length in streams. *Ecology* 89:3261-3267.
- Webb, P. W. 1982. Locomotor Patterns in the Evolution of Actinopterygian Fishes. *Am. Zool.* 22:329-342.
- Webb, P. W. 2004. Response latencies to postural disturbances in three species of teleostean fishes. *J. Exp. Biol.* 207:955-961.

- Wesner, J. S., E. J. Billman, A. Meier, and M. C. Belk. 2011. Morphological convergence during pregnancy among predator and nonpredator populations of the livebearing fish *Brachyrhaphis rhabdophora* (Teleostei: Poeciliidae). *Biol. J. Linn. Soc.* 104:386-392.
- Wohl, E. 2006. Human impacts to mountain streams. *Geomorphology* 79:217-248.
- Wohl, E. E. 2000. Inland flood hazards: human, riparian, and aquatic communities. Cambridge University Press, Cambridge, UK.
- Zandonà, E., S. K. Auer, S. S. Kilham, J. L. Howard, A. López-Sepulcre, M. P. O'Connor, R. D. Bassar, A. Osorio, C. M. Pringle, and D. N. Reznick. 2011. Diet quality and prey selectivity correlate with life histories and predation regime in Trinidadian guppies. *Funct. Ecol.* 25:964-973.
- Zelditch, M. L., D. L. Swiderski, H. D. Sheets, and W. L. Fink. 2004. Geometric morphometrics for biologists: a primer. Elsevier Academic Press, San Diego.

Table 3.1. Environmental data from collections sites. The first three letters of each site code indicate the watershed (Gua=Guanapo, Mad=Madamas, Par=Paria, and Qua=Quare). Sample size (n) for each collection correspond to the number of females over number of males, after excluding juveniles and curved individuals.

Site	n	Latitude	Longit.	Area (km ²)	Relief Ratio	Elong. Ratio	Canopy cover (%)	Pool Vol. (m ³)	Velocity (m/s)	CPUE	CPUE
Gua07	20/9	1183420	689820	0.61	0.33	0.41	80.33	27.04	0.02	0.00	5.00
Gua08	20/4	1183810	689850	2.85	0.29	0.71	89.79	610.74	0.04	0.00	2.00
Gua12	31/17	1184888	689101	1.38	0.25	0.52	84.45	313.25	0.03	0.00	2.00
Mad04	32/10	1188012	693194	1.76	0.36	0.71	71.84	125.22	0.12	16.25	1.25
Mad07	12/14	1188185	693766	0.62	0.34	0.50	72.5	17.49	0.01	18.00	0.00
Mad18	37/24	1188030	695400	0.29	0.21	0.65	90.3	21.93	0.06	9.00	3.00
Mad22	22/16	1188200	696900	0.64	0.13	0.59	84.32	34.55	0.03	10.00	0.00
Mad24	21/28	1188300	695500	5.57	0.23	0.92	76.43	237.86	0.08	11.00	0.00
Mad28	18/13	1188591	694295	6.12	0.24	0.84	91.78	1172.30	0.01	1.00	0.00
Par03	37/31	1191900	691630	3.12	0.18	0.91	86.95	130.24	0.03	12.67	2.67
Par04	38/30	1192052	691050	20.50	0.15	0.89	83.94	885.17	0.06	3.80	0.20
Par06	43/31	1191873	690546	2.00	0.1	0.84	84.8	113.43	0.14	4.50	0.50
Par10	34/19	1189434	690573	6.64	0.22	0.93	83.45	1398.28	0.11	13.00	5.00
Par11	35/18	1190775	689621	0.80	0.21	0.92	80.66	11.20	0.03	5.50	9.50
Par18	38/26	1191530	689200	1.00	0.12	1.08	86.01	179.15	0.04	5.50	9.50
Par21	15/16	1188765	691351	1.44	0.33	0.59	77.04	63.14	0.03	11.00	0.00
Par28	17/13	1189475	689818	1.10	0.1	0.79	78.45	16.49	0.08	7.00	0.00
Par29	16/18	1189150	691400	0.07	0.38	0.68	80.62	29.32	0.00	8.00	1.00
Qua08	24/9	1181310	697050	1.76	0.17	0.56	82.35	315.25	0.03	1.00	6.00
Qua09	19/22	1181000	697060	0.11	0.29	0.69	78.34	3.24	0.00	0.00	9.00
Qua10	24/8	1180670	696970	0.42	0.45	0.65	72.03	31.66	0.06	0.00	3.00
Qua20	15/10	1181000	696830	0.06	0.3	0.74	83.44	30.16	0.00	0.00	17.00

Table 3.2. Bivariate correlations between predictor variables. Correlation coefficients below diagonal, P-values above diagonal. P<0.05 and respective correlation coefficients in bold. AREA=watershed area, REL=Relief Ratio, ELO=Elongation Ratio, VOL=Pool volume (m³), VEL=water flow velocity (m/s), CAN=canopy cover (%), PRW = *Macrobrachium* spp. abundance (CPUE), RIV=*Rivulus hartii* abundance (CPUE).

	log AREA	REL	ELO	log VOL	log CAN	log VEL	log PRW	log RIV
log AREA	-	0.036	0.017	0.000	0.549	0.000	0.166	0.012
REL	-0.371	-	0.002	0.221	0.001	0.076	0.301	0.858
ELO	0.419	-0.529	-	0.068	0.223	0.074	0.033	0.369
log VOL	0.804	-0.222	0.326	-	0.089	0.013	0.893	0.189
log CAN	0.110	-0.572	0.222	0.305	-	0.647	0.282	0.302
log VEL	0.619	-0.318	0.320	0.434	-0.084	-	0.014	0.070
log PRW	0.251	-0.189	0.378	0.025	-0.196	0.430	-	0.069
log RIV	-0.438	0.033	0.164	-0.238	0.188	-0.325	-0.326	-

Table 3.3. MANCOVAs for the configurations of females early in pregnancy. F-ratios were estimated from Wilk's Λ . Partial η^2 values indicate effect size. Phylogenetic MANCOVA used Brownian motion on a phylogenetic tree with the original branch lengths proportional to geographic distances along the river channel. These branch lengths were transformed with Grafen (1989)'s method to proportionally reduce long branches. K indicates phylogenetic signal of the respective divergence vector (d). The correlations of each d with streamlining index (SI) were calculated from non-phylogenetic MANCOVAs. Significant p-values ($p < 0.05$) in bold.

Independent variable	Non-phylogenetic			Phylogenetic			Correlation of d with SI			
	df	F	p	Partial η^2	p	K	p (K)	R ²	Slope \pm SE	p
CS	1,7	9.277	0.047	0.96	0.305	1.238	<0.001	0.30	-4.50 \pm 1.43	0.005
log AREA	1,7	32.538	0.008	0.99	0.042	1.751	<0.001	0.17	-3.15 \pm 1.35	0.031
REL	1,7	1.954	0.313	-	0.341	0.701	0.020	<0.01	-0.01 \pm 2.44	0.995
ELO	1,7	5.027	0.106	-	0.212	1.190	<0.001	0.18	-3.86 \pm 1.62	0.027
log CAN	1,7	5.554	0.093	-	0.152	0.507	0.260	0.10	-4.56 \pm 2.48	0.081
log VOL	1,7	1.969	0.311	-	0.389	0.610	0.058	0.17	-5.00 \pm 2.17	0.032
log VEL	1,7	2.007	0.305	-	0.327	0.431	0.539	<0.01	-0.49 \pm 2.37	0.839
log PRW	1,7	51.500	0.004	0.99	0.014	1.911	<0.001	0.20	-3.39 \pm 1.34	0.020
log RIV	1,7	16.879	0.020	0.98	0.022	1.690	<0.001	0.08	2.75 \pm 1.60	0.102
Watershed	3,21	1.677	0.212	-	0.511	-	-	-	-	-

Table 3.4. MANCOVA for the configurations of males. Explanation of variables as in Table 3.2.

Independent variable	Non-phylogenetic				Phylogenetic			Correlation of <i>d</i> with SI		
	df	F	p	Partial η^2	p	K	p (K)	R ²	Slope \pm SE	p
CS	1,7	2.940	0.203	-	0.587	1.641	< 0.001	0.53	6.29 \pm 1.26	< 0.001
log AREA	1,7	5.177	0.102	-	0.293	1.296	< 0.001	0.35	4.17 \pm 1.19	0.002
REL	1,7	14.756	0.025	0.97	0.023	1.195	< 0.001	0.16	5.15 \pm 2.31	0.038
ELO	1,7	7.781	0.060	-	0.175	1.175	< 0.001	0.33	-5.07 \pm 1.52	0.003
log CAN	1,7	2.602	0.232	-	0.306	0.370	0.868	0.01	-3.34 \pm 2.98	0.277
log VOL	1,7	5.178	0.102	-	0.099	0.646	0.043	<0.01	3.75 \pm 4.12	0.374
log VEL	1,7	0.236	0.947	-	0.975	0.364	0.885	0.25	-5.84 \pm 2.04	0.010
log PRW	1,7	18.663	0.018	0.98	0.050	1.628	< 0.001	0.36	4.86 \pm 1.36	0.002
log RIV	1,7	2.003	0.305	-	0.280	0.555	0.172	<0.01	1.97 \pm 2.36	0.414
Watershed	3,21	2.451	0.081	-	0.327	-	-	-	-	-

Figure 3.1. Sampling localities. Upper panel shows the Northern range of Trinidad island. Lower panel zooms the rectangle in the upper panel, showing sites and watersheds. Par=Paria, Mad=Madamas, Gua=Guanapo, Qua=Quare.

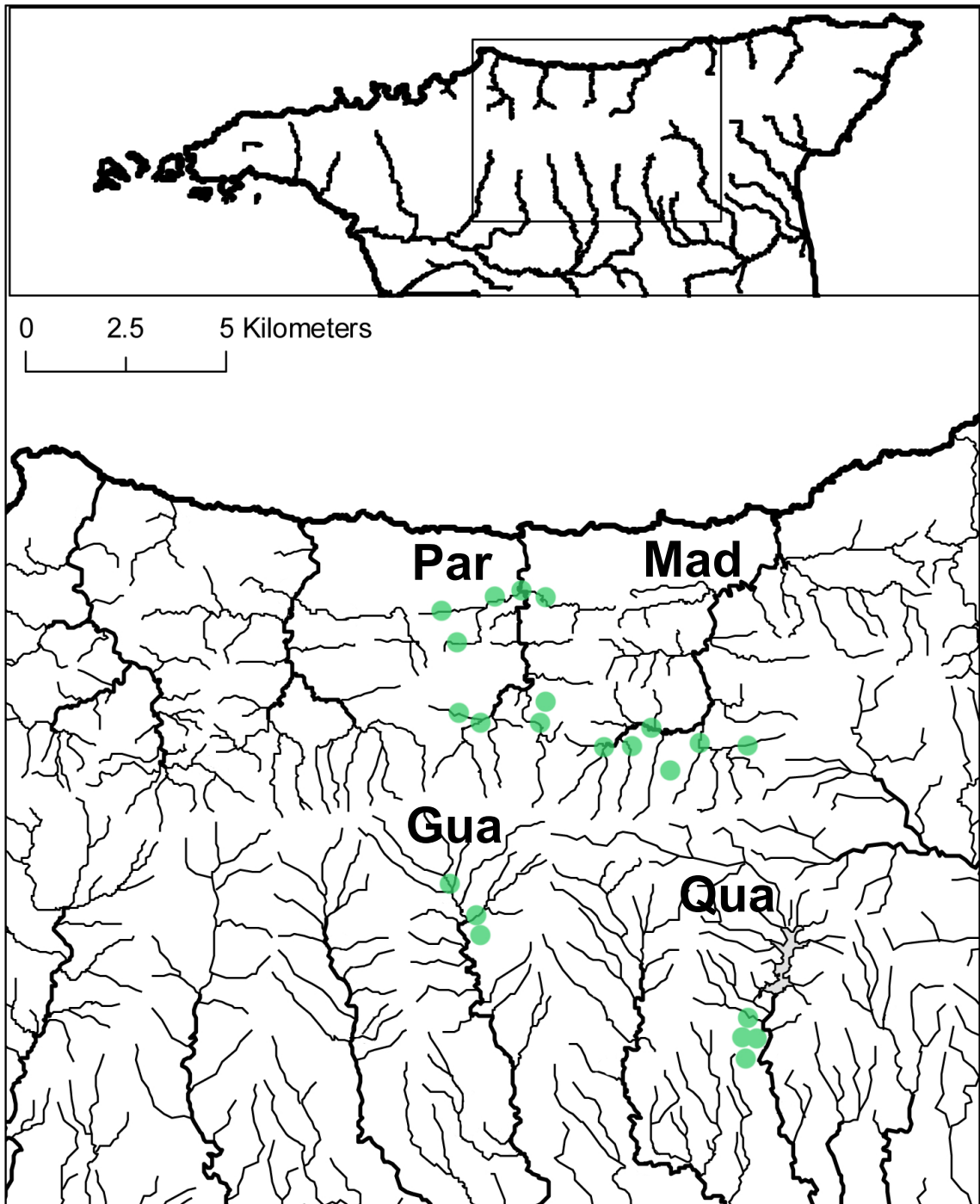


Figure 3.2. Shape variation estimated with MANCOVAs. A. Females early in pregnancy. B. Males. C. Females late in pregnancy. Only significant effects shown. Shapes are represented with thin-plate splines, grey and black outlines are the extreme low- and high-values, respectively. Arrows inside outlines are proportional to the magnitude and direction of shape differences. Scatterplots beside splines show the relationship between each factor and the canonical vector of divergence. Shape differences have a four-fold magnification.

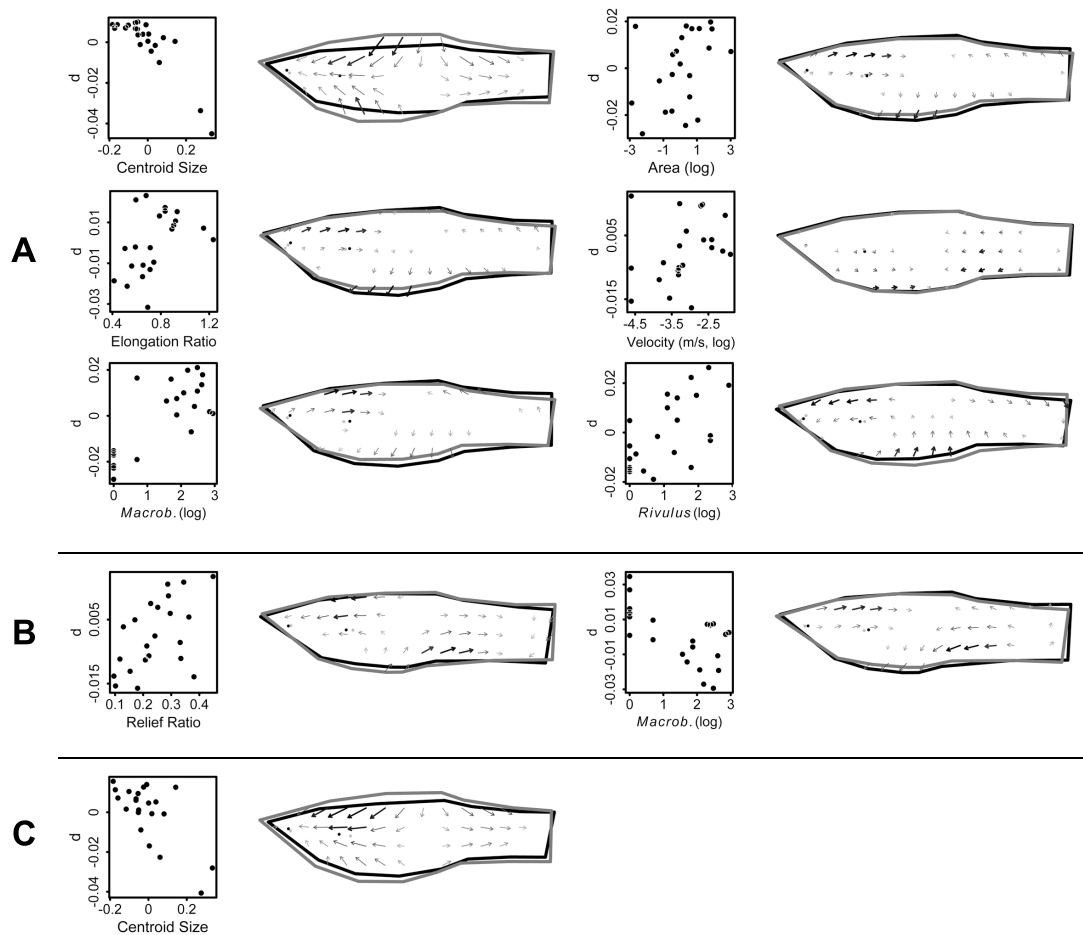
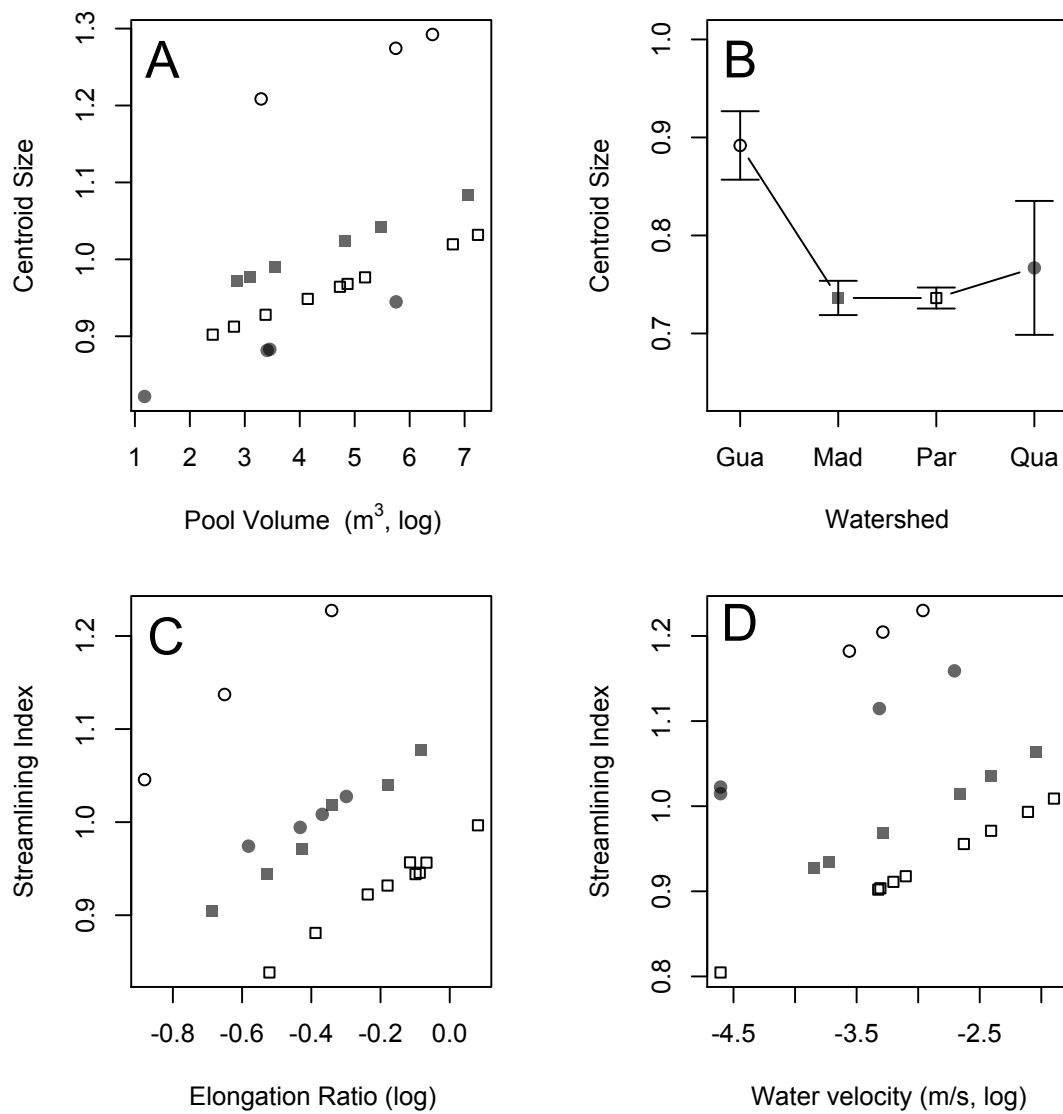
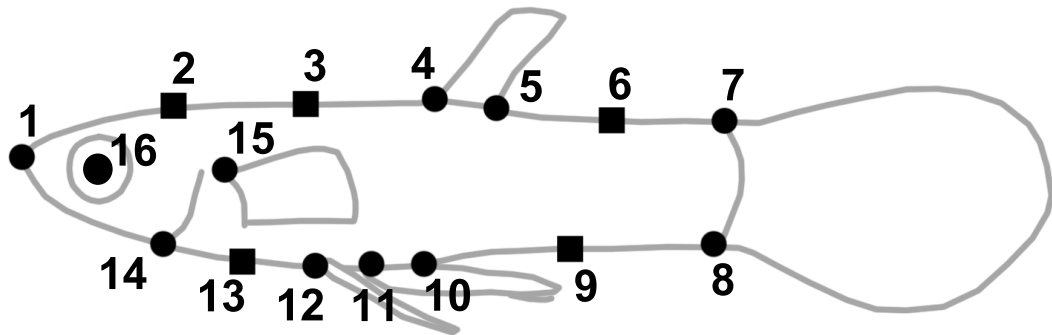


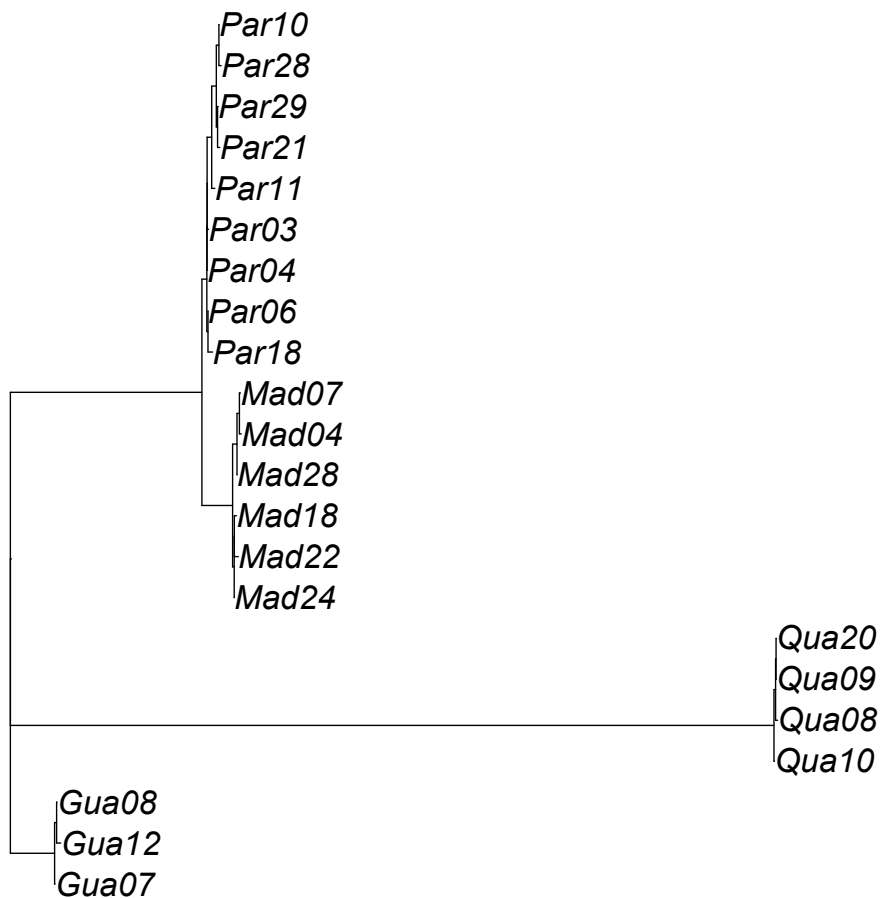
Figure 3.3. Body size and shape variation estimated with regressions. A and B are regressions with body size of females and males, respectively. C and D are regressions with Streamlining Index of females and males, respectively, Symbols in all panels indicate watershed origin, following arrangement of panel B.



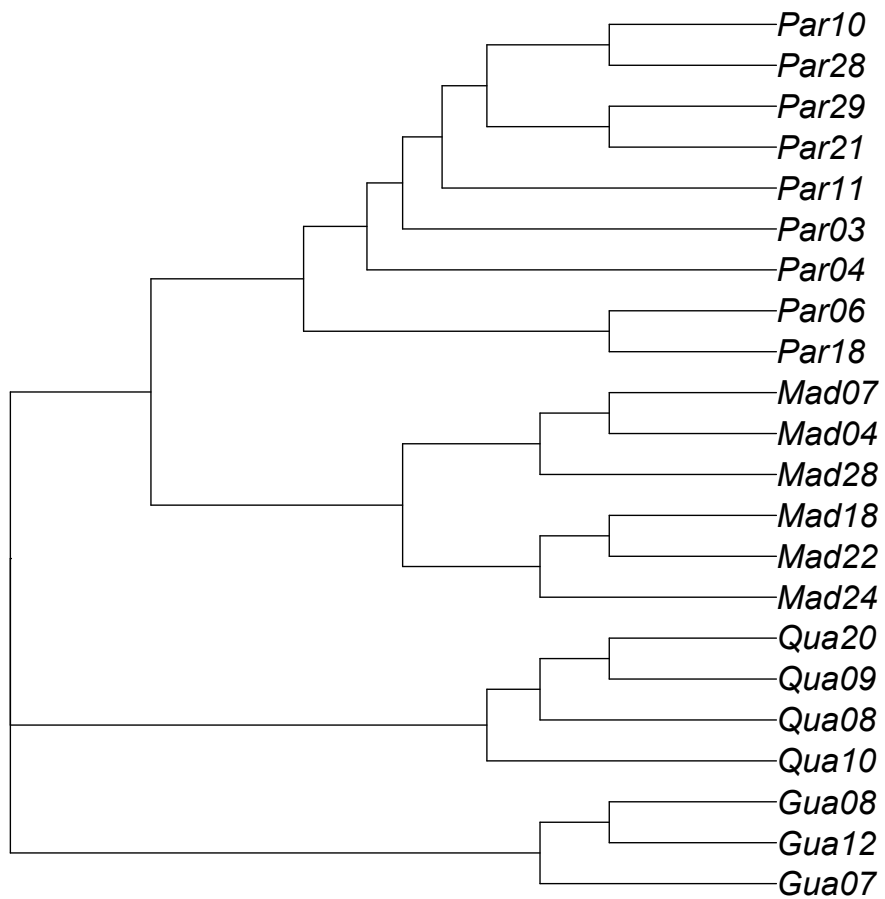
Appendix 3.1. Position of landmarks (circles) and semilandmarks (squares) in lateral view of guppies. Numbers correspond to the following positions: (1) tip of the snout; (2-3) semilandmarks along dorsal midline between (1) and (4); anterior (4) and posterior (5) terminus of dorsal-fin base; (6) semilandmark along dorsal midline between (5) and (7); dorsal (7) and ventral (8) terminus of caudal-fin base; (9) semilandmark along ventral midline between (8) and (10); posterior (10) and anterior (11) terminus of anal fin (gonopodium in males); (12) anterior terminus of pelvic-fin base; (13) semilandmark along ventral midline between (12) and (14); (14) bottom of head at junction of border of opercle and body; (15) anterior terminus of pectoral fin; and (16) eye center.



Appendix 3.2. Phylogeny derived from river network hierarchy. Branch lengths proportional to geographical distances between sites along the streambed. First three letters of site name correspond to watershed names (Par=Paria, Mad=Madamas, Qua=Quare, and Gua=Guanapo).



Appendix 3.3. Phylogeny depicted in Appendix 3.2. after transformation of branch lengths using Grafen's (1989) power transformation method. This transformation was performed to reduce the negative influence of long branches in calculations.



Appendix 3.4. Candidate set of regression models for Centroid Size (CS) and Streamlining Index (SI) of females (F, early and late in pregnancy) and males (M). Only best supported models ($\Delta AIC_C=4$) shown. Models indicated with a regression equation, where AREA= watershed AREA (km², log), REL= REL Ratio, ELO= ELO Ratio, CAN= percentage of CAN cover (log), VOL=Pool VOL (m³, log), VEL=water flow VEL (m/s, log), Macrobrachium=CPUE abundance of *Macrobrachium* spp. (log), and RIV=CPUE abundance of *RIV hartii* (log). When CS was the dependent variable, it was not included as a covariate. For each model the following features are listed: number of parameters (k), logarithm of its Maximal Likelihood (lnL), small-sample Akaike Information Criterion (AIC_C), correlation coefficient (R^2), Akaike weights (w, the percentage of variation explained by each model), and Evidence Ratio (ER, the number of times that each model is worse than the best supported model of each set).

Sex	Stage Pregnancy	Model	k	lnL	AIC _c	R ²	w	ER
F	-	CS~VOL+Watershed	6	28.0	-38.41	0.75	38.9	1.0
F	-	CS~AREA+Watershed	6	26.7	-35.76	0.72	10.4	3.8
F	-	CS~VEL+Watershed	6	26.6	-35.55	0.72	9.3	4.2
F	-	CS~Watershed	5	24.6	-35.42	0.66	8.7	4.5
F	Early	SI~CS+ELO+Watershed	7	28.7	-35.42	0.61	36.7	1.0
F	Early	SI~CS+REL+Watershed	7	28.3	-34.66	0.60	25.1	1.5
F	Early	SI~CS+Watershed	6	24.8	-32.05	0.45	6.8	5.4
F	Early	SI~CS+VEL+Watershed	7	26.8	-31.59	0.54	5.4	6.8
F	Late	SI~CS+REL+Watershed	7	23.5	-25.02	0.47	33.4	1.0
F	Late	SI~CS+Watershed	6	20.8	-23.97	0.33	19.7	1.7
F	Late	SI~CS+CAN+Watershed	7	21.9	-21.82	0.39	6.7	5.0
F	Late	SI~CS+PRW+Watershed	7	21.8	-21.70	0.39	6.3	5.3
F	Late	SI~CS+VEL+Watershed	7	21.6	-21.29	0.38	5.2	6.5
M	-	CS~Watershed	5	30.5	-47.18	0.43	29.5	1.0
M	-	CS~VOL+Watershed	6	31.6	-45.50	0.48	12.8	2.3
M	-	CS~VEL+Watershed	6	30.8	-44.07	0.45	6.3	4.7
M	-	CS~ELO+Watershed	6	30.8	-44.00	0.45	6.0	4.9
M	-	CS~AREA+Watershed	6	30.8	-43.90	0.44	5.7	5.2
M	-	CS~PRW+Watershed	6	30.7	-43.84	0.44	5.6	5.3
M	-	CS~REL+Watershed	6	30.6	-43.56	0.43	4.8	6.1
M	-	CS~CAN+Watershed	6	30.5	-43.35	0.43	4.4	6.8
M	-	CS~Watershed ^a	6	30.5	-43.33	0.43	4.3	6.9
M	-	CS~Watershed ^b	6	30.5	-43.33	0.43	4.3	6.9
M	-	CS~RIV+Watershed	6	30.5	-43.33	0.43	4.3	6.9
M	-	SI~CS+VEL+Watershed	7	28.6	-35.28	0.72	60.7	1.0

Notes: Twelve adaptive models were tested for each dependent variable (DV): DV~CS+Watershed, DV~CS+AREA+Watershed, DV~CS+REL+Watershed, DV~CS+ELO+Watershed, DV~CS+CAN+Watershed, DV~CS+VOL+Watershed, DV~CS+VEL+Watershed, DV~CS+Macrobrachium+Watershed, DV~CS+RIV+Watershed, DV~CS+AREA+REL+ELO+Watershed, DV~CS+CAN+VOL+VEL+Watershed, DV~CS+Macrobrachium+RIV+Watershed, DV~CS+AREA+REL+ELO+CAN+VOL+VEL+Macrobrachium+RIV+Watershed. The models of character evolution tested were ordinary least squares (OLS, non-phylogenetic multiple regression), Pagel's (RegPagel), and Ornstein–Uhlenbeck (RegOU). Most of the resulting best supported models were OLS. The exceptions are marked with superscripts: a) RegPagel, with phylogenetic signal = 1.30E-17; and b) RegOU, with phylogenetic signal = 1.30E-17.

Concluding Remarks

In this dissertation I studied complex adaptive processes that involved multiple environmental and phenotypic interacting variables. In the first two chapters I used the *Gambusia* female system to test whether processes commonly observed at the intraspecific level were also present at the interspecific level. The first chapter was an interspecific analysis in body shape of females of 20 species of *Gambusia*. The main question here was whether predation and flow regimes explain body shape in these females. The main result was that, as predicted by biomechanical theory and in agreement with microevolutionary studies, female *Gambusia* showed relatively larger caudal regions in high-predation sites. This indicates that there is a continuum from micro- to macroevolutionary scales in the effect of predation on body shape of these females. At least in this system, microevolutionary patterns of phenotypic divergence were useful to predict macroevolutionary patterns of body shape variation. Unexpectedly, flow explained very little of the wide range of variation observed. As later explored in Chapter 3, flow may be a complex variable not always related to shape. One of the novel aspects of this analysis was that it accounted for pregnancy condition and for phylogenetic relatedness. The results show the importance of taking pregnancy into account because the way shape changed through pregnancy was related with predation regime, that is, pregnancy reduced the magnitude of phenotypic divergence between females from contrasting predation regimes.

Further studies may try to assess whether the different ways to be pregnant may explain the differences in locomotive performance of livebearing species (Ghalambor et al. 2004). The phylogenetic analysis had the challenge to deal with multivariate shape data (in the case of geometric morphometrics). Here I developed new software that allows such analysis. Our observation here agrees with the generality that phylogenetic signal is typically strong in shape variation (Blomberg et al. 2003), indicating that accounting for phylogeny in shape analysis of hierarchically structured samples should be the default analytical strategy. Also regarding methods, we observed that different currently widespread methods to measure body shape were not interchangeable, but rather complementary. Further functional shape analysis should complement the currently ubiquitous application of geometric morphometrics with other estimations of shape that are more directly connected with functional aspects of shape.

The second chapter I also explored the continuation of adaptive divergence from micro- to macroevolutionary scales, this time with the life history of *Gambusia* females as the focal traits. In contrast to the analysis of shape, and despite theoretical predictions and previous observations at the microevolutionary level, predation or flow did not predict the wide range of life history observed in this genus. This negative results may be a consequence of the large sensitivity of life history traits to environmental conditions, perhaps in these case some conditions that we did not measure such as food availability or conspecific density. Nevertheless, this analysis showed interesting novel aspects,

such as the observation of incipient matrotrophy in few species and the first data on the life history of several species (some of them with a delicate conservation status). Moreover, we observed a rare life history phenomenon with unexplored theoretical implications: allometric coefficients vary with predation regime. Specifically, species from High-Predation sites increased in gonadal size at a higher rate with growth. This phenomenon deserves further theoretical and empirical investigation.

One interesting avenue for future studies on the *Gambusia* system would be to simultaneously analyze both sexes at intra- and interspecific scales. The data collected here, in addition to data from previously published studies (Langerhans et al. 2007; Langerhans and Reznick 2010) are a readily available source for such analysis.

In the third chapter I focused more specifically in flow regime to explain body shape of male and female guppies. The broad theme of this question was the study of adaptation to a disturbance. We observed a correlation between body shape and geomorphological features of the watersheds, but the results varied between sexes. A stronger predictor of shape was, interestingly, the abundance of prawns and *Rivulus*. That is, two biotic factors (the abundance of two competing or mildly predatory species) were more important for explaining body shape variation than abiotic factors associated with flow regime. This result pays homage to Darwin, who always favored biotic over abiotic factors when explaining evolutionary patterns in nature.

Literature Cited

- Blomberg, S. P., T. Garland, Jr, and A. R. Ives. 2003. Testing for phylogenetic signal in comparative data: behavioral traits are more labile. *Evolution* 57:717-745.
- 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*). *The American Naturalist* 164:38-50.
- Langerhans, R. B., M. E. Gifford, and E. O. Joseph. 2007. Ecological speciation in *Gambusia* fishes. *Evolution* 61:2056-2074.
- Langerhans, R. B., and D. N. Reznick. 2010. Ecology and evolution of swimming performance in fishes: predicting evolution with biomechanics. Pp. 200-248 in P. Domenici, and B. G. Kapoor, eds. *Fish Locomotion: an etho-ecological perspective*. Science Publishers, Enfield.