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Banet, Amanda Inez

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Evolutionary Diversification of Reproductive Modes in Livebearing Fishes

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

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in

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by

Amanda Inez Banet

December 2009

Dissertation Committee:
Dr. David. N. Reznick
Dr. Derek A. Roff
Dr. Douglas L. Altshuler

The Dissertation of Amanda Inez Banet is approved:

Committee Chairperson

University of California, Riverside

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The text of this dissertation, in part, is a reprint of the material as it appears in:

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Banet, A. I. & Reznick, D. N. (2008). Do placental species abort offspring? Testing an assumption of the Trexler-DeAngelis model. *Functional Ecology* **22**, 323-331.

The introduction of this dissertation contains section 21.5 from the book chapter listed in the former citation, which I wrote and researched. The listed co-authors wrote other sections of the chapter and assisted with editing. Chapter 1 is a reprint in full of the latter cited paper. The co-author, David N. Reznick, directed and supervised the research, which forms the basis for this dissertation.

ABSTRACT OF THE DISSERTATION

Evolutionary Diversification of Reproductive Modes in Livebearing Fishes

by

Amanda Inez Banet

Doctor of Philosophy, Graduate Program in Biology
University of California, Riverside, December 2009
Dr. David N. Reznick, Chairperson

Placental matrotrophy has evolved independently in many lineages, which is indicative of strong selection. Several ad-hoc hypotheses and one mathematical model have been proposed to explain its evolution, but little experimental data exists to support these claims. Recent studies have attempted to find correlates between placental matrotrophy and other life history traits, but the only common correlate found was a reduction in reproductive allocation (RA). Here I report on three studies. The first two focus on the Trexler-DeAngelis model for the evolution of matrotrophy. In chapter one, I use closely related placental and non-placental species from the northern clade of *Poeciliopsis* to test an assumption imperative to the model, that placental species abort a subset of developing offspring in low food conditions. The results show no evidence of abortion due to food level. Instead, placental species appear to be tethered to a brood once initiated, and sacrifice body condition to maintain reproduction when resources are restricted. However, an alternative explanation for these results is that the pattern of resource allocation is a function other life history traits, rather than placentation alone. Chapter two distinguishes between these alternatives, by performing a similar experiment on the southern clade of *Poeciliopsis*, which has the opposite relationship between life history traits and placentation seen in the northern clade. The results mirror those from the northern clade, indicating reproductive mode, rather than life history, dictates the pattern of resource allocation. This further adds to the difficulties of explaining the evolution of the placenta within the constraints of the Trexler-DeAngelis model. The third study characterizes locomotor consequences related to differences in reproductive allocation using the Trinidadian Guppy (*Poecilia reticulata*), because placentation is correlated with a reduction in reproductive allocation. Females with the highest RA were less streamlined, had lower escape velocities, and had to work harder during prolonged swimming. In low predation natural habitat, these same females were restricted to habitat with slower moving water, while females with lower RAs were able to use faster flows, which are thought to be preferred feeding grounds. All high predation fish used slow moving water, irrespective of RA.

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INTRODUCTION

The direct link between reproduction and fitness makes studies of reproductive strategies particularly relevant in evolutionary biology (Fisher 1930). Optimality theory predicts that life-history traits such as age and size at maturity, number and size of offspring, and growth and reproductive investment will be linked through a series of trade-offs, and will be selected to maximize fitness (Roff 1992, Stearns 1992). In addition, we often see multiple independent origins of reproductive strategies, which is indicative of strong selection. Because of this, the reproductive patterns we see in nature are generally assumed to be the result of strong selection to optimize reproductive output, rather than a result of random processes such as genetic drift.

Reproductive strategies vary in their costs and benefits (Tinkle 1969). This variation, in combination with ecological conditions, can influence how selection acts on an organism. Thus, understanding the costs and benefits associated with different reproductive strategies can give insight on their evolution and maintenance. For example, viviparity has arisen at least 100 separate times in squamate reptiles (Blackburn 1981). Such a case lends itself to comparative studies that can shed light on contributing selective factors. A well documented cost associated with retaining young is decreased locomotor performance, which can result in increased susceptibility to predators or an increased energy expenditure (Ghalambor et al. 2004, Plaut 2002, Seigel et al. 1987, Shine 1980, Van Damme et al. 1989, Walker et al. 2005, Wu et al. 2004). It has long been noted that the presence of viviparity in squamates is correlated with colder climates

(Sergeev 1940), and subsequent studies have focused on what benefit may be conferred by retaining young in colder climates. These studies suggest that faster development time and higher offspring quality are two such benefits (Brown & Shine 2004, Shine 1995, Shine & Bull 1979). Assuming these costs and benefits fully describe trade-offs associated with livebearing, viviparity will evolve when the fitness gain due to faster embryo development and higher offspring quality outweighs any fitness loss that may be incurred due to reduced locomotion. However, once viviparity evolves, subsequent evolution may act to reduce its associated costs.

Variation within livebearers

The goal of this dissertation is to investigate the evolution and maintenance of variation within livebearers, specifically the evolution of placental matrotrophy and correlated life history traits. To do this, I study fish from the family Poecillidae, which contains multiple independent origins of placental matrotrophy, each of which has closely related species that are non-placental.

Timing of maternal provisioning for livebearing species follows a continuum. At one end of the continuum is lecithotrophy (non-placental), where organisms are essentially like egg-layers that retain their eggs for the course of gestation. The eggs at fertilization are fully yolked and no additional nutrients are delivered to the young post-fertilization. At the other extreme is matrotrophy, where the female starts with tiny eggs at fertilization and allocates a substantial amount of resources throughout gestation. This can be done by means of oophagy, histophagy, or placentation (Trexler & DeAngelis

2003), the latter of which is the method of nutrient transfer focused on in this study. While the evolution of viviparity itself has been extensively studied, fewer studies have looked at the evolution of matrotrophy, and the body of literature available does not provide a satisfactory explanation for the ultimate cause of its occurrence. This gap in knowledge is surprising, given that matrotrophic viviparity has evolved independently in at least 24 clades of viviparous vertebrates (Blackburn 1992), and is the sole mode of reproduction for all placental mammals.

The placenta: a complex trait

Mossman defined placental viviparity as "any intimate apposition or fusion of the fetal organs to the maternal or paternal tissues for physiological exchange." (Mossman 1937). Largely, this fusion of the fetus occurs with maternal, rather than paternal, cells. The placenta was recognized as early as 1822 to consist of both maternal and fetal tissue (Home 1822). Maternal blood vessels bring blood to the offspring, which is completely dependent on the mother for obtaining nutrients and disposing of waste. In the mammalian placenta, which has been described in more detail than any other, the placenta suppresses immunological interactions, which could otherwise be fatal to a developing embryo. Additionally, zygote derived placental cells secrete hormones which affect maternal metabolism, modify maternal arterial vasculature, and maintain the uterus in the correct physiological condition for the zygote (Georgiades et al. 2002, Haig 1993).

Mossman (1937) noted the great structural diversity of the placenta that exists across taxa. In the early part of the 19th century, this was "to be considered as the means employed by nature to prevent the whole system respecting animals from being thrown into confusion, by preventing any two different genera from breeding together" (Home 1822). This diversity potentially complicates uncovering universal explanations for the evolution of the matrotrophy. However, Reznick et al. (2002) point out that all livebearers with extensive post-fertilization provisioning must face similar demands, and thus must have similar adaptations.

Of additional interest, placental matrotrophy can be viewed as a complex trait. Darwin explained the evolution of "organs of extreme complication and perfection," or complexity, in terms of gradual, beneficial steps. He suggested that examination of a trait in different levels of complexity would give insight on its evolution (Darwin 1872). To study the evolution of complexity, it is preferable to study a trait where intermediate steps have been retained, and there is a varying degree of complexity in closely related species. Reznick et al. (2002) argue that the placenta fulfills this need. They point out that over 50 loci have been characterized that regulate the development of the placenta, and argue that this, taken in combination with its multiple phenotypic adaptations, comprises a complex trait. Additionally, they used a relaxed molecular clock approach to estimate the time needed for three highly matrotrophic species of fish in the genus *Poeciliopsis* to develop extensive post-fertilization provisioning. Their estimates were 2.36 million years ago and 0.75 million years ago, depending on the locality of the clade they were from, which is commensurate to estimates for the eye, which historically has been the archetype organ for studying the evolution of complexity. Reznick et al. propose that the range of variation present in closely related extant placental species allows researchers a unique opportunity to study the evolution of complexity, stating “this range of variation in *Poeciliopsis* is comparable to finding a single genus that has three independent origins of elaborate eyes including congeners that have either no eyes, or eyes in various intermediate stages of evolution.”

HYPOTHESES FOR THE EVOLUTION OF THE PLACENTA

The convergent evolution of matrotrophy, with its multiple independent origins, is indicative of strong selection (Losos et al. 1998). This argues that the placenta is either in itself an adaptation, or the result of many counter-adaptations, as it is unlikely that a complex trait would numerous times be the outcome of drift. Hypotheses for the evolution of placental matrotrophy fall into two categories: adaptive hypotheses and conflict hypotheses. Adaptive hypotheses assume that there is some external factor such as predation or resource availability acting as a selective pressure that promotes the evolution of the placenta (Trexler & DeAngelis 2003). Conflict hypotheses suggest that parent-offspring conflict in-utero drives its evolution, and rather than being an adaptation itself, is the result of multiple counter-adaptations (Crespi & Semeniuk 2004, Haig 1993, Haig 1996, Zeh & Zeh 1996). While these hypotheses are presented separately for simplicity, I do not mean to suggest that they are mutually exclusive. It is possible that they may act in concert, or that one may be a necessary precursor for the other.

Ecological Hypotheses

While ecological explanations for the evolution of life history traits have historically been commonplace, there are few environmental explanations in the literature for the evolution of matrotrophy. Sir William Turner, an anatomist and contemporary of Charles Darwin, thought it an impossible question to answer. While he suggested several factors that may have contributed to its occurrence and morphology, including speed of the organism, gestation time, organism size, and offspring number, he could not distinguish any single factor that clearly affected the structure or presence of the placenta (Magee 2003, Turner 1876). More recently, several ad-hoc hypotheses and one model have proposed that certain ecological conditions will favor the evolution of the placenta. These hypotheses are discussed below.

Resource availability hypotheses

Matrotrophic females start reproduction with small eggs and provide additional nutrient investment to offspring throughout development, allowing a female to spread investment into offspring over a greater period of time when compared to strictly or predominantly lecithotrophic reproductive modes. Such differences in allocation patterns can be studied under the framework of life-history adaptations and, just as differences between capital and income breeders (Drent & Daan 1980, Houston et al.). This approach may hold the key to identifying a possible advantage of matrotrophy.

Trexler and DeAngelis (2003) developed a combination of analytical and simulation models to investigate what resource conditions would favor the matrotrophic reproductive mode over the lecithotrophic mode. The analytic model examines the reproductive success of each reproductive mode during a single reproductive event, assuming a size-number tradeoff in initial egg number. For simplicity, it also assumes that terminal offspring size is the same for both reproductive strategies. From a set amount of starting resources, a lecithotroph produces fewer, fully yolked eggs. Since the matrotroph starts with a smaller egg, she can produce more eggs initially, which will then need additional resource investment throughout gestation. If resources are consistently abundant during the gestation period, the matrotroph thus has the potential for producing a greater brood size than the lecithotroph. However, if food levels are low or unpredictable, producing a large number of eggs may be counterproductive for the matrotrophic female because she risks having insufficient resources to nourish all offspring and may thus lose the entire brood. This leads to a key assumption in the model: when resources become scarce, the matrotroph is assumed to have the ability to abort and resorb energy from some offspring within the brood. The simulation model expands on the analytical model by examining lifetime reproductive success of each reproductive mode across a range of resource levels and embryo resorption capabilities. It takes growth, storage, and schedule of reproduction into account. Overall, the model finds that matrotrophy is most likely to evolve in habitats where abundant resources are consistently available.

This is supported by Thibault and Schultz's description of the habitats of several species live-bearing fishes in the genus *Poeciliopsis* (Thibault & Schultz 1978). They found that *P. monacha*, a lecithotrophic fish with no post-fertilization provisioning, is found in the harshest environments, where resource availability fluctuates wildly. *P. prolifica*, a highly matrotrophic species, is found in predictable high resource localities. *P. lucida*, which has an intermediate level of placentation, is found in environments that nestle between that of *P. prolifica* and *P. monacha* in terms of resource availability and predictability.

The ability to abort and resorb offspring expands the conditions under which a matrotroph would have higher lifetime reproductive success: without the ability to abort, the predicted habitat range for species with a matrotrophic reproductive mode is narrowed only to areas with the highest, most predictable resource levels.

Locomotor Costs Hypothesis

The evolutionary transition from egg-laying to livebearing implies an increase in the length of time that a female is physically bound to her developing offspring. While the female is emancipated from a nest, she must still carry the developing offspring with her during daily activities, including foraging and predator avoidance. Studies in a variety of taxa have shown that egg retention and livebearing reduce locomotor performance (Bauwens & Thoen 1981, Ghalambor et al. 2004, Plaut 2002, Shine 1980, Van Damme et al. 1989, Wu et al. 2004). Since matrotrophic species have a smaller initial egg size, the physical burden they carry for a given number of offspring, particularly at the early stages of pregnancy, is smaller than that of lecithotrophic species. Thibault and Schultz (1978) suggested that this resulted in a ‘streamlining’ of matrotrophic species, thus reducing the locomotor costs of internal development. Several lines of evidence add support to their hypothesis.

The size of the reproductive package a female carries has been linked to locomotor performance in the guppy, *Poecilia reticulata*. Guppies are lecithotrophic; their embryos lose dry mass over the course of development. However, guppy embryos have nearly a 4-fold increase in wet mass between fertilization and birth because of an increase in the water content of developing embryos (Ghalambor et al. 2004). Ghalambor et al. (2004) examined different components of the escape response, or C-start, in pregnant female guppies. They found that stage of pregnancy (and thus the size of the reproductive package) correlated well with maximum velocity, distance traveled, turning angle, and mean rotational velocity during an escape, with females at later stages of pregnancy and larger reproductive packages showing impairment. They also found that guppies from high predation localities, which have a higher reproductive allocation on average (e.g. Reznick & Endler 1982), performed better in many aspects of the escape response than low predation guppies; however, they only did so when they were carrying embryos that were in earlier stages of development. High-predation guppies experienced a more rapid decline in velocity and distance traveled during the escape as the pregnancy progressed, suggesting a greater locomotor cost of reproduction for high predation females due to the larger embryo size at later stages of reproduction.

Walker et al. (2005) verified the assumption that a faster escape response decreases the likelihood of predation during predator-prey interactions in a lab setting. They examined likelihood of predator evasion using the guppy and a natural predator, the pike cichlid (*Crenicichla alta*), and found that predator evasion was positively correlated with two locomotor performance variables: rapid rotational velocity in the early stage of the escape response and ‘rapid tangential acceleration,’ a composite variable including net distance traveled, maximum velocity, and maximum acceleration (Walker et al. 2005). This result thus confirms that the reduced performance associated with pregnancy will increase susceptibility to predation.

Locomotor costs of pregnancy are not limited to predator-prey interactions, however. Routine swimming, such as that used to maintain position against water flow velocity, can also be deleteriously affected during pregnancy. Studies of pregnant *Gambusia affinis* revealed a significant decrease in critical swimming speed (a measure of endurance) over the course of pregnancy (Plaut 2002). Body mass and cross sectional area of the female increased as the pregnancy progressed. Surprisingly, the study did not find any change in tailbeat amplitude or frequency when swimming a fixed speed as the pregnancy progressed. Tailbeat frequency and/or amplitude would be expected to increase if the decrease in critical swimming speed was due to the physical burden of pregnancy, which would result in increased drag and reduced flexibility, indicating that a female was working harder to swim a fixed speed. The author suggests that the locomotor cost is thus caused by a physiological impairment rather than a physical burden during pregnancy.

Finally, a study of life-history traits in another fish family, the Zenarchopteridae, revealed that in the genus *Dermongenys*, matrotrophic species produced fewer, larger offspring than lecithotrophs, while in the genus *Nomorhampus*, the trend was reversed:

matrotrophic species produced more, smaller offspring (Reznick et al. 2007). The only consistent life-history correlate with matrotrophy was reproductive allocation, which is lower in matrotrophic species in both genera. The cost of locomotion was not directly addressed in this study, but a reduced cost of locomotion is implied in matrotrophic zenarchopterids if decreased reproductive allocation is correlated with increased locomotory performance.

The studies discussed above point to both matrotrophy being consistently correlated with reduced reproductive allocation and, consequently, with a reduced burden of pregnancy via improved locomotor performance. It is noteworthy that no study conducted to date has addressed how the consequences of differences in reproductive allocation play out in a natural setting.

Conflict Hypotheses

Conflict hypotheses are those that suggest parent-offspring conflict during gestation drives the evolution of matrotrophy (Charalambous et al. 2003, Coall & Chisholm 2003, Crespi & Semeniuk 2004, Haig 1993, Trivers 1974, Zeh & Zeh 1996). In sexual organisms, mother and offspring are not identical, which may cause natural selection acting on the mother to oppose that acting on the offspring (Haig 1993). In terms of fitness, a female will fare best when all of her offspring survive and reproduce. For her offspring, this may not be the case. If an offspring gains a fitness benefit by getting more resources, and this benefit outweighs the inclusive fitness costs that may be incurred via its siblings or mother, then any trait that allows an offspring to gain more resources will be beneficial and spread in the population (Haig 1993). This conflict is magnified when a female mates multiply, because the coefficient of relatedness between siblings is reduced, reducing inclusive fitness costs. Thus, parent-offspring conflict is expected to result in adaptations that allow the offspring to elicit more resources from the parent than is in the best interest of the parent (Crespi & Semeniuk 2004, Haig 1993, Trivers 1974). This conflict can also be viewed as sexual conflict, because a male will benefit if his offspring elicits more resources.

While parent-offspring conflict is often viewed as a post-parturition occurrence, Trivers recognized that viviparity provides a potential arena for this conflict to take place during gestation in 1974. The interface of maternal and fetal tissues is not a defined line, but rather an intermingling of mother and offspring. This intimate connection between mother and offspring via the placenta has been suggested to be less than the harmonic relationship previously thought. The interface has been likened to a battlefield, where the maternal tissue can be seen as a defender against an invading trophoblast (Haig 1993). Modifications of the maternal arterial vasculature and hormone secretion by the fetal placental tissue may be an offspring's attempt to manipulate the mother into allocating more resources than is in her best interest. Resultantly, an arms race may occur where offspring are selected to manipulate the mother, and females counter this with an adaptation to resist such manipulation. Crespi and Semeniuk (2004) suggest that in this light, viviparity promotes antagonistic coevolution, genomic divergence, and postzygotic isolation. Haig (1993) is quick to point out that conflict does not imply that either mother or offspring would benefit from a severe change in their relationship during development.

He uses the analogy of a tug-of-war, where each sides pulls, building tension in a rope that connects them. If one side suddenly quits, the other side collapses.

Despite the growing amount of theory on conflict in-utero, there is little empirical evidence to directly support these claims. This, in part, may be due to difficulties in designing experiments that properly detect conflict (see Rowe et al. 2003). However, a growing body of circumstantial supporting evidence does exist, which cumulatively provides support for conflict hypotheses for the evolution of the placenta.

FOCUS OF SPECIFIC CHAPTERS

This dissertation contains three chapters, which touch on all of the above-mentioned hypotheses. In chapter one, I compare the response of closely related matrotrophic and lecithotrophic species (*Poeciliopsis prolifica* and *Poeciliopsis monacha)* to a reduction in food availability, to test the assumption of the Trexler-DeAngelis model that matrotrophic species have the ability to abort and resorb offspring in low food conditions. In chapter two, I repeat a similar experiment on another closely related pair of matrotrophic and lecithotrophic species, *Poeciliopsis turneri* and *Poeciliopsis gracilis*. Repeating the experiment on another pair strengthens the study because it enabled me to determine whether the results of the first study were due to species-specific traits, or whether they could be attributed to the presence or absence of the placenta. Chapter two also elucidates differences in resource provisioning (somatic growth/maintenance vs. reproduction) between the two reproductive modes, and discussing the implications of this on conflict theory. In chapter three, I examine how the link between varying levels of reproductive allocation, locomotor performance, and habitat use, follow with a discussion of the implications of this relationship on the evolution of the placenta.

REFERENCES:

Bauwens, D. & Thoen, C. (1981). Escape tactics and vulnerability to predation associated with reproduction in the lizard *Lacerta vivipara*. *Journal of Animal Ecology* **50**, 733-743.

Blackburn, D. (1981). An evolutionary analysis of vertebrate viviparity. *American Zoologist* **21**, 963A.

Blackburn, D. (1992). Convergent evolution of viviparity, matrotrophy, and specializations for fetal nutrition in reptile and vertebrates. *American Zoologist* **32**, 313-321.

Brown, G. P. & Shine, R. (2004). Effects of reproduction on the antipredator tactics of snakes (Tropidonophis marii, Colubridae). *Behavioral Ecology and Sociobiology* **56**, 257-262.

Charalambous, M., Ward, A. & Hurst, L. (2003). Evidence for a priming effect on maternal resource allocation: implications for interbrood competition. *Proceedings of the Royal Society* **270**, S100-S103.

Coall, D. & Chisholm, J. (2003). Evolutionary perspectives on pregnancy: maternal age at menarche and infant birth weight. *Social Science and Medicine* **57**, 1771-1781.

Crespi, B. & Semeniuk, C. (2004). Parent-offspring conflict in the evolution of vertebrate reproductive mode. *American Naturalist* **163**, 635-653.

Darwin, C. *The Origin of Species* (eMallBooks, 1872)

Drent, R. & Daan, S. (1980). The prudent parent: energetic adjustments in avian breeding. *Ardea* **68**, 225-252.

Fisher, R. A. *The Genetical Theory of Natural Selection* (Clarendon Press, Oxford, 1930)

Georgiades, P., Ferguson-Smith, A. C. & Burton, J. G. (2002). Comparative developmental anatomy of the murine and human definitive placentae. *Placenta* **23**, 3-19.

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

Haig, D. (1993). Genetic conflicts in human pregnancy. *Quarterly Review of Biology* **68:4**, 495-532.

Haig, D. (1996). Gestational drive and the green-bearded placenta. *Evolution* **93**, 6647-6551.

Home, E. (1822). On the placenta. *Philosophical Transactions of the Royal Society of London* **112**, 401-407.

Houston, A. I., Stephens, P. A., I.L., B., Harding, K. C. & McNamara, J. M. Capital or income breeding? A theoretical model of female reproductive strategies. *Behavioral Ecology* **18**, 241-250.

Losos, J. B., Jackman, T. R., Larson, A., de Queiroz, K. & Rodriguez-Schettino, L. (1998). Contingency and determinism in replicated adaptive radiations of island lizards. *Science* **279**, 2115-2118.

Magee, R. (2003). Sir William Turner and his studies on the mammalian placenta. *ANZ Journal of Morphology* **73**, 449.

Mossman, H. W. (1937). Comparative morphogenesis of the fetal membranes and accessory uterine structures. *Contributions to Embryology* **26**, 129-146.

Plaut, I. (2002). Does pregnancy affect swimming speed in female mosquitofish, *Gambusia affinis? Functional Ecology* **16**, 290-295.

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

Reznick, D., Meredith, R. & Collette, B. B. (2007). Independent evolution of complex life history adaptations in two families of fishes, live-bearing halfbeaks (Zenarchopteridae, Beloniformes) and Poeciliidae (Cyprinodontiformes). *Evolution* **61**, 2570-2583.

Reznick, D., Mateos, M. & Springer, M. (2002). Independent origins and rapid evolution of the placenta in the fish genus *Poeciliopsis*. *Science* **298**, 1018-1020.

Roff, D. *The Evolution of Life Histories* (Routledge, Chapman, and Hall, New York, 1992)

Rowe, L., Cameron, E. & Day, T. (2003). Detecting sexually antagonistic coevolution with population crosses. *Proceedings of the Royal Society* **270**, 2009-2016.

Seigel, R. A., Huggins, M. M. & Ford, N. B. (1987). Reduction in locomotor ability as a cost of reproduction in gravid snakes. *Oecologia* **73**, 481-485.

Sergeev, A. M. (1940). Reasearches in the viviparity of reptiles. *Moscow Society of Naturalists* 1-34.

Shine, R. (1980). "Costs" of reproduction in reptiles. *Oecologia* **46**, 92-100.

Shine, R. (1995). A new hypothesis for the evolution of viviparity in reptiles. *American Naturalist* **145**, 809-823.

Shine, R. & Bull, J. (1979). The evolution of live-bearing in lizards and snakes. *American Naturalist* **113**, 905-923.

Stearns, S. C. *The Evolution of Life Histories* (Oxford University Press, New York, 1992)

Thibault, R. & Schultz, R. J. (1978). Reproductive adaptations of viviparous fishes (*Cyprinodontifomes: Poeciliidae)*. **32:2**, 320-333.

Tinkle, D. (1969). The concept of reproductive effort and its relation to the evolution of life histories of lizards. *American Naturalist* **103**, 501-516.

Trexler, J. & DeAngelis, D. (2003). Resource allocation in offspring provisioning: an evaluation of the conditions favoring the evolution of matrotrophy. *American Naturalist* **162**, 574-585.

Trivers, R. (1974). Parent-offspring conflict. *American Zoologist* **14**, 249-264.

Turner, W. *Lectures on the Comparative Anatomy of the Placenta. First Series*. 1876)

Van Damme, R., Bauwens, D. & Verheyen, R. (1989). Effect of relative clutch mass on sprint speed in the lizard *Lacerta vivipara*. *Journal of Herpetology* **23**, 459-461.

Walker, J. A., Ghalambor, C., Griset, O. L., McKenney, D. & Reznick, D. (2005). Do faster starts increase the probability of evading predators? *Functional Ecology* **19**, 808-815.

Wu, W., Meijer, O. G., Lamoth, C., Uegaki, K., van Dieen, J. H., Wuisman, P., de Vries, J. & Beek, P. J. (2004). Gait coordination in pregnancy: transverse pevic and thoracic rotations and their relative phase. *Clinical Biomechanics* **19**, 480-488.

Zeh, J. & Zeh, D. (1996). The evolution of polyandry I: intragenomic conflict and genetic incompatibility. *Proceedings of the Royal Society* **263**, 1711-1717.

CHAPTER 1

DO PLACENTAL SPECIES ABORT OFFSPRING? TESTING AN ASSUMPTION OF THE TREXLER-DEANGELIS MODEL

SUMMARY: We investigate how resource level affects reproduction in matrotrophic (*Poeciliopsis prolifica*) and lecithotrophic (*Poeciliopsis monacha)* fishes. One of our goals was to test an assumption of the Trexler-DeAngelis model for the evolution of matrotrophy, which was that matrotrophic species can adjust litter size by aborting offspring in low food conditions. Our more general goal was to elucidate other differences between the reproductive modes. Both species have superfetation and an approximately 30-day development time. Females of each species were assigned to high or low food availability for 30 days, or one gestation period. Any young born during that time interval would have initiated development before the initiation of the experiment. If embryos are aborted, then this would be seen as a reduction in brood size in the low food treatment relative to the high food treatment within this period. Our results suggest *P. monacha* responds to low food by sacrificing reproduction to maintain lipids, while *P. prolifica* maintains reproduction at the expense of lipids. Neither species showed a significant reduction in offspring number over the course of the experiment, suggesting that these species do not abort offspring in low food conditions.

INTRODUCTION

Understanding variation in life history traits is a central focus of research in evolutionary biology because these traits play such a direct role in determining fitness. A central feature of life history traits is that they are often functions of one another; these functional relationships define the tradeoffs that we think play a fundamental role in shaping the way life histories evolve (Roff 1992, Stearns 1992). For example, fecundity is often a function of some other aspect of the organism, such as body size. As a consequence, there can be a trade-off between current and future fecundity that is mediated by the way the current investment in reproduction affects growth, future body size, and hence future fecundity (Roff 1992, Stearns 1992). Fecundity is also strongly influenced by the mode of reproduction, such as egg-laying versus livebearing, and hence is likely to tilt the balance in the favor of the evolution of one or the other of these modes of reproduction (Tinkle 1969). Tinkle observed, for example, that livebearing lizards only produce one clutch of eggs per year, while egg layers can produce four or more clutches per season, so livebearing implies a large potential loss of fecundity. He suggested that livebearing may most often evolve in those populations of lizards that normally only produce a single clutch of eggs per year because they would suffer little or no loss of fecundity. More generally, the reproductive mode we see is presumably an adaptation that maximizes fitness in the face of these trade-offs.

Many hypotheses have been proposed to explain the evolution of livebearing and many empirical research programs have been devoted to the testing and development of these hypotheses (Shine 1980, Shine 1983, Qualls 1997, Hodges 2004, Shine 2004).

However, a less visually apparent, but perhaps equally important difference in reproductive mode has to do with timing of provisioning. In viviparous species, timing of maternal provisioning ranges from strict lecithotrophy (yolk-feeding) to extreme matrotrophy (mother-feeding). In lecithotrophic species, females produce a fully provisioned egg that is fertilized then retained inside of the female throughout development. The only post-fertilization investment involves gas exchange and waste disposal. In matrotrophic species, the egg size at fertilization is greatly reduced and does not contain sufficient resources to sustain growth and development. Most nutrients are provisioned after fertilization, by means of oophagy, histophagy, or placentation (Turner 1947, Wourms, Grove & Lombardi 1988, Trexler & DeAngelis 2003). Lecithotrophy is generally considered to be the ancestral state (Reznick, Mateos & Springer 2002), however, matrotrophy has evolved independently in at least 24 clades of viviparous vertebrates (Blackburn 1992). The shift between matrotrophy and lecithotrophy represents a distinct change in the timing of provisioning, making when and where resources for reproduction come from important in determining whether a reproductive bout is successful. The multiple independent origins of matrotrophy are suggestive of strong selection (Losos et al. 1998). While mammalian reproduction is the most well known example of matrotrophy, it exists in a wide range of taxa, including reptiles, fishes, insects, and plants (Turner 1947, Lloyd 1980, Wourms et al. 1988, Farley 1998, Jerez & Ramirez Pinilla 2001, Chen & Caswell Chen 2004). Despite this, very little theoretical or empirical work has addressed the factors that have shaped the evolution of matrotrophy (Trexler & DeAngelis 2003). Currently, only one model exists in the literature, and neither its assumptions nor its predictions have been empirically tested.

Trexler and DeAngelis (2003) used a combination of analytical and simulation techniques to produce the first non-verbal ecological model for the evolution of matrotrophy, focusing on what environmental conditions would favor matrotrophy over lecithotrophy, given a set of assumptions. They assumed that matrotrophy increases the number of offspring a female could have per reproductive bout. This is because a lecithotroph allocates all or most energy to an egg in a short interval of time before fertilization, while a matrotroph starts with smaller, less expensive eggs and allocates throughout gestation. From the same starting costs, a matrotroph can make more eggs initially and, given enough resources during the gestation period, will carry more embryos to term (Trexler & DeAngelis 2003). Additionally, they find that the conditions that favor the evolution of matrotrophy are more easily met if a female can diminish brood size via abortion, should resource levels drop below that required to sustain the brood. They consider a range of values for ability to resorb energy from these aborted embryos, and model short and long-term lipid stores for each strategy. Finally, the model assumes that terminal offspring size is the same for both strategies, irrespective of resource availability.

The assumption that matrotrophs can abort embryos in the Trexler-DeAngelis model is an important one. Based on the model, without this ability, matrotrophy will be favored only in an extremely narrow range of conditions, where food availability is consistently equal to, or higher than that needed to meet maintenance and reproduction needs. Any deviation from consistently high resource availability results in a catastrophic loss of fecundity because resources will be spread too thin and all offspring in a given brood will be lost. More generally, abortion of embryos has been suggested to confer a fitness advantage when optimal brood size is unknown or when the mother can identify the fitness potential of offspring during development and abort those with low potential (Lloyd 1980, Diamond 1987, Stearns 1987, Kozlowski & Stearns 1989).

However, empirical data on the advantage, or even existence, of embryo abortion in this context is lacking. For example, Borowsky and Kallman (1976) suggest that stress-induced abortion occurs in the platyfish, *Xiphophorus maculatus* because field-caught females had approximately twice the interbrood interval as lab-reared females. They interpreted this difference as indicative of abortion in the field-caught females. However, without common garden conditions, it is impossible to conclusively determine the cause of this difference. Trexler (1997) found that sailfin mollies, *Poecilia latipinna*, which are either lecithotrophic or facultatively matrotrophic, had fewer offspring at parturition than fertilization, regardless of whether they were reared on high or low food levels, which again suggests that some offspring were aborted between fertilization and birth. Meffe and Vrijenhoek (1981) concluded that abortion and resorption of embryos did not occur in several species of starved poeciliid fishes with a range of reproductive modes.

Seed plants have the equivalent of a placenta since extensive maternal provisioning occurs after the seed is fertilized. Lalonde and Roitberg (1989) looked for seed abortion due to decreased resource level in the dioecious plant *Cirsium arvense*.

They defined abortion as an ovary that displayed pericarp development (ripened fruit wall), but that did not contain a healthy embryo. They found that abortion did occur, but the level of abortion was consistent between seeds in high and low resource conditions. Instead, significant differences in the level of abortion were found between clones, suggesting that abortion was due to non-viable gene combinations, rather than resource availability. Manzur et al. (1995) found that in humans, spontaneous abortion of 1 to 3 embryos occurred in 52.6% of women carrying triplets.

Our goal is to test the assumption of the Trexler-DeAngelis model that matrotrophic females have the ability to abort offspring under conditions of low food. Here we do so by determining how matrotrophs and lecithotrophs differ in their response to food levels. We predict that lecithotrophs will not abort offspring in response to reduced food availability because nutrients are pre-packaged before fertilization. In contrast, we predict that matrotrophs will abort offspring in response to reduced rations because they require a continuous supply of nutrients to sustain developing young. We examined closely related matrotrophic and lecithotrophic species of *Poeciliopsis* fishes to determine whether embryo abortion occurs in these respective reproductive modes.

METHODS

The genus *Poeciliopsis* consists of small, livebearing fishes with internal fertilization. This genus contains reproductive strategies that range from lecithotrophy to extensive matrotrophy, with well-developed placentas (Turner 1940, Reznick et al. 2002). Because of this diversity of modes of reproduction, they are an ideal group to study matrotrophy and the evolution of post-fertilization provisioning. We used *Poeciliopsis prolifica* (matrotrophic) and *Poeciliopsis monacha* (lecithotrophic) to investigate differences between reproductive modes. These species are more closely related to one another than each would be to another species with the same reproductive mode in the genus. A phylogenetic reconstruction of the evolution of maternal provisioning in this genus shows that *P. prolifica* represents one of the three independent origins of extensive matrotrophy in *Poeciliopsis* (Reznick et al. 2002). The offspring of the *P. prolifica* in our experiment display an eight-fold increase in dry mass between fertilization and birth (Pires, McBride & Reznick 2007). This increase is associated with an elaboration of maternal and embryonic tissues, which function together as the equivalent of a placenta (Turner 1940). In contrast, the embryos of *P. monacha* lose approximately 40% of their dry mass during development, presumably reflecting the costs of metabolism and anabolism (Thibault & Schultz 1978, Reznick et al. 2002). This degree of weight loss is comparable to what is seen in the mass of a freshly laid egg versus newly born embryo in egg-laying species (Wourms 1981) and hence is interpreted as lecithotrophy. Both species have superfetation, or the ability to carry multiple broods in different stages of development, making them virtual conveyor belts of offspring. This trait allowed us to look at the effect of a given experimental treatment on embryos that are in different stages of development. Both species have the ability to store sperm for an indeterminate amount of time (Winge 1937, Turner & Snelson 1984, Constanz 1989) allowing us to keep females in isolation during the course of a short-term experiment without remating.

To determine the effect of food on developing embryos in matrotrophic and lecithotrophic species, we used a factorial design with factors being food level (high/low) and reproductive mode (matrotrophic/lecithotrophic) as fixed factors. The gestation period for any given litter is approximately 30 days (Pires and Reznick, unpublished data), so the effect of food reduction on developing offspring will be reflected in offspring born within 30 days of the initiation of the treatment. For each female, we recorded 15 days of reproduction on high food levels to establish a baseline, or “before” rate of reproduction. Females were then randomly assigned either high or low rations. This treatment lasted 30 days, or one gestation period. A key to our design is that when a female begins her treatment, she will contain multiple litters of young that are already developing. All of the young born during the course of the experiment will be from eggs that were fertilized before the experiment began. Young that were in an advanced stage of development will be born early in the experimental period while those that were in earlier stages will be born progressively later. Because each litter was exposed to reduced maternal food availability for different durations of development, we will also be able to ascertain not only if abortion occurs, but also whether or not the stage of development of the embryo at the time of food deprivation affects whether or not it will be aborted. Thus, a reduction in brood size in the low food group as compared to the high food group is interpreted as the abortion of developing embryos due to reduced resource level. A subset of females was kept in the experiment for 30 additional days. Offspring born during this second 30 day interval were from litters that were initiated after experimental food treatments began and reflect the added effect of food level on the number of eggs that are fertilized. *P. monacha* sample sizes were 16 and 19 females for high and low food groups, respectively. *P. prolifica* had 22 females per treatment group. See Figure 1.1 for a schematic of the design.

The *P. prolifica* were the second and third laboratory generation derived from adults collected from *el Palillo* river in 2004. *P. monacha* were from a laboratory population that was derived from adults collected from the Rio Fuerte drainage and that had been in laboratory culture since 2001. Females of similar size and age were reared with mature males in 38-liter community tanks and were fed ad libitum with liver paste in the morning and brine shrimp in the evening. Once actively reproducing, females were isolated in 8-liter aquaria and fed high food rations (just under average ad libitum levels for the lab population). Females were kept on quantified food for one gestation period, or approximately 30 days, before the study began, to ensure that broods used in the analysis were initiated after quantified food rations began. We then collected 15 days of baseline data, followed by the 30-day treatment.

We used Hamilton micropipettes to ensure accurate allocation of food. For *P. prolifica*, high food levels were 40 μL per meal, and low food levels were 15 μL per meal. For *P. monacha*, high food levels were 50 μL per meal, and low food levels were 15 μL per meal. Initially, all high food treatments were 50 μL, but it was discovered that *P. prolifica* females were not eating all rations, so the level was reduced to prevent detrimental effects in water quality due to overfeeding. *P. monacha* are larger on average, which probably accounts for the differences in the rate of food consumption.

We measured length and mass of females on day 1 and day 30 of the treatment. Females were preserved at the end of day 30. All offspring born after females were isolated in 8-liter aquaria were preserved immediately after birth. Fish were euthanized using an overdose of MS-222 and preserved in 5% formaldehyde. The main dependent variable of interest for determining whether abortion occurs was offspring number. However, matrotrophic females may respond to low food in a number of unpredicted ways, including extending the gestation period, producing smaller, leaner offspring, or by sacrificing lipid stores to provision young. Thus, we measured female dry mass, female fat content, offspring dry mass, and offspring fat content following the methods in Reznick & Yang (1993) to provide a more complete picture of the reproductive biology of each species. A repeated measures ANCOVA was used to analyze offspring number, with species and food treatment as independent variables, and female size as a covariate. The repeated measures were the 15 days immediately preceding the treatments, days 1-15 of treatment, and days 16-30 of treatment. A similar ANOVA was used to analyze offspring dry mass and offspring fat content, where the covariate was not needed. A two-tailed t-test was used to analyze changes in interbrood interval, reproductive allocation, degree of superfetation, female dry mass, female fat content, and number of developing young. Some analyses below omit females either because she did not give birth in a given time interval, or because she was part of the subset that was allowed to continue in the experiment for 60 days. Some broods were excluded from offspring dry mass and fat content analyses because they were inadvertently destroyed during processing. These deletions are noted in the degrees of freedom, reported below.

RESULTS

Female Size: Low food females weighed approximately three quarters of their high food counterparts at the end of the experiment, despite no differences initially (Repeated measures ANOVA: *P. monacha* (lecithotrophic): $F_{(1,33)}$=91.184, p<0.001, *P. prolifica* (matrotrophic): $F_{(1,42)}$=96.724, p<0.001), indicating that food levels were sufficient enough to invoke a response (Table 1.1).

Offspring Number: All treatment groups showed a decline in fecundity between the pre-treatment and treatment period. This may be due to a net decline in food availability after the fish were isolated in 8-liter tanks and kept on quantified rations. No difference in offspring number was detected between food treatments for either species for the 30 day treatment period (Table 1.1: Fig 1.2: *P. monacha* (lecithotrophic): $F_{(2,31)}$=0.003, p=0.997, *P. prolifica* (matrotrophic): $F_{(2,40)}$=0.337, p=0.716, using females size as a covariate). *P. prolifica* does show a slight, non-significant decline in offspring production in the low food group relative to the high food group towards the end of the 30 day treatment period (Fig. 1.2b, partial-Eta-squared 0.017). The absence of a difference in fecundity suggests an absence of the abortion of developing embryos in both species. We did not perform formal analyses of the reproductive data for the subset of females that were retained in the experiment for an additional 30 days because of the small sample sizes (3-5 per treatment group) but the trends show that fecundity declines in the low food treatments of both species, likely because fewer offspring are initiated per litter. The rate of decline in

P. prolifica was more gradual than in *P. monacha; P. monacha* ceased reproduction by the end of the 60 day interval.

Offspring Mass: Response in dry mass of offspring did not differ between treatment groups for lecithotrophic *P. monacha* (Table 1.1; Fig 1.3a: $F_{(2,26)}=0.807$, p=0.457). In matrotrophic *P. prolifica*, dry mass of offspring from high food females increased over the course of the experiment. In low food *P. prolifica* females, dry mass of offspring decreased (Fig 1.3b: $F_{(2,40)}=0.8.304$, p=0.001). This decline in offspring size in the low food treatment implies that *P. prolifica* females are relying on food consumption to support the growth of developing young rather than relying solely on fat stores.

Offspring Fat Content: The composition of offspring born during the course of the experiment was not affected by food level. Offspring from high and low food females showed a similar percentage of lipids within each species (Table 1.1: *P. monacha* (lecithotrophic): $F_{(2,24)}=0.031$, p=0.966, *P. prolifica* (matrotrophic): $F_{(2,36)}=1.306$, p=0.284).

Interbrood Interval: Interbrood interval was analyzed by taking the difference in days between the last full interval before treatments began and the last full interval before the experiment ended, and then comparing this number between high and low food groups. Interbrood interval did not differ between the food groups for either species (Table 1.1: *P. monacha* (lecithotrophic): $t_{(28)}=-0.013$, p=0.990, *P. prolifica* (matrotrophic): $t_{(41)}=-1.132$, p=0.264), indicating that neither species responds to low food by lengthening development time. Differences between species in interbrood interval is due to *P. prolifica* having more developing broods of young and hence a shorter interbrood interval than *P. monacha*.

Female Lipids: At the end of the experiment, *P. monacha* (lecithotrophic) showed no significant difference in composition between food groups (Table 1.1: $t_{(24)}$=1.336, p=0.194). In contrast, high food *P. prolifica* (matrotrophic) had a significantly higher proportion of lipid content than low food *P. prolifica* (Table 1.1: $t_{(32)}$=4.714, p=<0.001).

Reproductive Allocation (RA): Low food *P. monacha* (lecithotrophic) females showed a significant reduction in reproductive allocation (reproductive dry mass/ total dry mass) at the end of the 30 day treatment compared to high food females (Table 1.1: $t_{(24)}$=4.901, p<0.001). Reproductive allocation in *P. prolifica* (matrotrophic) was not statistically different between food groups (Table 1.1: $t_{(33)}$=-0.630, p=0.533).

Superfetation: At the end of the 30 day treatments, lecithotrophic *P. monacha* showed a significant decrease in the number of developing litters in the low food treatment compared to the high food treatment (Table 1.1: $t_{(24)}$=3.633, p=0.001). Food level did not have an impact on the number of developing litters in matrotrophic *P. prolifica* (Table 1.1: $t_{(33)}$=0.015, p=0.988).

Number of Developing Young: Developing young present in females at the end of the 30 experiment would be ones who initiated development after the experiment began. Dissection of lecithotrophic *P. monacha* females revealed that low food females had fewer developing young than high food females (Table 1.1: $t_{(24)}=3.624$, $p=0.001$). There was no difference between high and low food groups in matrotrophic *P. prolifica* (Table 1.1: $t_{(33)}=1.453$, $p=0.156$). Stage zero eggs (yolked eggs with no embryonic development) were not used in this analysis because it is difficult to determine litter size before eggs are fully yolked and fertilized.

DISCUSSION

We did not find evidence of abortion in matrotrophic species. All offspring born in the course of the 30-day treatments were initiated before the treatments began. If abortion occurs, then low food groups should have a reduction in fecundity as compared to the high food control groups. However, neither species showed significant differences in offspring number between the high and low food treatments. If this is a general property of the Poeciliidae, then the conditions that favor the evolution of matrotrophy under the Trexler-DeAngelis model are much narrower, and matrotrophy will be favored only when food availability is consistently equal to or higher than that needed to meet maintenance and reproduction needs. Deviation from consistently high resource availability will result in resources being spread too thin and all offspring in a given brood will be lost.

There was a hint of a reduction in the fecundity of the low food treatment of matrotrophic *P. prolifica* towards the end of the treatment period, which would suggest that they might have some ability to abort embryos, but only ones that are early in development. If *P. prolifica is* indeed capable of such embryo abortion, their ability is limited and a much larger experiment would be required to perceive it as statistically significant. Alternatively, it may be that abortion is a strategy that only occurs in more extreme conditions. Even restricting abortion to more extreme conditions would reduce the scope of conditions that favor the evolution of matrotrophy.

Trexler and DeAngelis modeled an organism that lacked superfetation and that produced offspring of constant size. The reality of the mode of reproduction in our study organisms leaves them with an enlarged spectrum of responses to a reduction in food availability. Matrotrophic species responded to a switch to low food by producing smaller offspring in those broods that were initiated before food level dropped. As expected, developing broods in the lecithotroph were buffered from the reduction in food level, and showed no decrease in mass. The decrease in mass in *P. prolifica* is due to an overall reduction in offspring size, rather than a reduction in lipid content. This reduction is consistent with findings in another matrotroph, *Heterandria formosa*. Reznick, Callahan & Llauredo (1996) found that *H. formosa* responded to low food by producing smaller offspring of similar composition, and suggested that this may be due to an inability of matrotrophs to allocate fat reserves to developing offspring. The two species did not respond to reduced food by modifying the rate of development of their young since the interbrood intervals of the high and low food treatments were the same.

Producing smaller offspring is likely to be maladaptive in low resource environments. The production of larger offspring has been predicted to be adaptive in resource poor environments (Smith & Fretwell 1974, Sibly & Calow 1983, McGinley, Temmer & Geber 1987, McGinley & Charnov 1988, Laurie & Brown 1990, Lalonde 1991, Charnov, Downhower & Brown 1995, Einum & Fleming 2004), and many studies have provided empirical evidence in a variety of species to support the predictions (Ferguson & Fox 1984, Glicwicz & Guisande 1992, Parichy & Kaplan 1992). Hassall et. al (2006) found that under favorable conditions, maternal fitness in the grasshopper *Chorthippus brunneus* was highest when females produced a large clutch with small eggs. However, under poor conditions, maternal fitness was highest when each clutch consisted of few, large offspring. Hutchings (1991) found that in brook trout, larger offspring had higher survival in low food conditions, but this advantage was reduced in high resource conditions. Given this evidence, a matrotroph that produces smaller offspring in low food conditions will likely have a selective disadvantage compared to a lecithotroph that does not reduce offspring size in low food conditions.

Reznick et al. (1996) found that lecithotrophic species (*Poecilia reticulata*, *Priapicthys festae*) responded to a reduction in food availability by producing larger offspring. Bashey (2002) showed that this size increase is likely to represent adaptive phenotypic plasticity because of the fitness advantage that these larger babies have in low food environments. The fact that matrotrophic species seem to respond to a sudden reduction in food availability in the opposite fashion suggests that this response represents a non-adaptive constraint associated with the evolution of matrotrophy, which would again restrict the conditions that favor the evolution of matrotrophy. Lecithotrophic *P. monacha* is expected to show a similar adaptive plasticity in low food conditions as *P. reticulata* and *P. festae*. A formal test of this plasticity is in progress.

Dissection data of females at the end of the experiment give insight into how the food level would affect broods that were initiated during the treatment regime. Low food *P. monacha* (lecithotrophic) had reduced reproductive allocation, degree of superfetation, and litter size, but not a reduction in lipid stores. Low food *P. prolifica* (matrotrophic) showed a reduction in the percent of lipids, but not in the number of developing broods of young, litter size, or in reproductive allocation. This suggests that *P. monacha* responds to low resource conditions by defending maintenance at the expense of reproduction. *P. prolifica* instead reduces its reserves in favor of maintaining reproduction.

If our results represent a general trend for all matrotrophs, then they indicate a reduction in the range of conditions where a matrotroph could out-compete a lecithotroph compared to that suggested by the Trexler-DeAngelis model. A maladaptive response in terms of offspring size makes some sense in the light of Trexler and DeAngelis' prediction that matrotrophy evolved in a consistent, high resource environment. If this is the case, then selection for such an adaptive response to low food should be relaxed. Alternatively, if matrotrophy requires a consistent level of food availability to produce appropriate sized offspring, then it may represent a constraint that limits the ability of matrotrophic species to persist in variable environments. This conclusion is consistent with Thibault and Schultz's (1978) narrative description of the habitats of *P. monacha*, *P. prolifica*, and *Poeciliopsis lucida*. Lecithotrophic *P. monacha* is found in the harshest environments, where there are “dramatic seasonal fluctuations” in temperature, water levels, light intensity, and resource availability. Matrotrophic *P. prolifica* is found in more specialized habitats, often deep, permanent pools, where food resources are at consistently high levels. *P. lucida*, which has an intermediate level of matrotrophy, is found in environments of intermediate resource availability and predictability (Thibault & Schultz 1978).

Marsh-Matthews and Deaton (2006) looked at the effects of food level on the reproduction of *Gambusia geiseri* also with the goal of evaluating aspects of the Trexler-DeAngelis model. *G. geiseri* has been termed an incipient matrotroph, meaning that while it is primarily lecithotrophic, it may provision very low levels of nutrients after fertilization if resource levels are high enough. They reared fish on high and low food treatments beginning approximately 44 days before exposure to males. They found that high food females had larger brood sizes, larger embryos, and a higher rate of nutrient transfer (measured by the injection or radiolabeled nutrients). They found no difference in the probability of embryo abortion, observed directly via dissection, between treatments. The increase in embryo size was attributed to incipient matrotrophy. This result is interpreted as support for the Trexler-DeAngelis model since they predicted that matrotrophy is more likely to evolve in the presence of high resource availability. It differs from the model because they are reporting on phenotypic plasticity, while Trexler and DeAngelis were predicting the circumstances that favor the evolution of the trait.

Both the Trexler (1997) study on sailfin mollies, and the Marsh-Matthews and Deaton (2006) study looked for evidence of abortion in facultative matrotrophs that had been reared on high and low levels of food availability prior to the initiation of the litters that were the dependent variables in their experiment; both studies found evidence for abortion, but it was independent of food level. Our study instead quantifies the potential abortion caused by variation in food availability while the young are developing. The differences between the experimental designs can be thought of as possible scenarios that a pregnant female may encounter, and likely represents a small portion of the variation that might be encountered in nature.

The overall contrast in the life histories of *P. monacha* and *P. prolifica* are reminiscent of a more general trend that we see in life history evolution. Lecithotrophic *P. monacha* responds to resource restriction by maintaining itself at the expense of reproduction while matrotrophic *P. prolifica* responds by maintaining reproduction at the expense of maintenance. Elsewhere it has been shown that *P. prolifica* is younger at first reproduction and produces offspring at a higher rate (Thibault & Schultz 1978). *P. prolifica* thus has the kind of life history that is predicted to evolve in environments with high extrinsic mortality (Charlesworth 1994). If this were a general property of species with matrotrophy, then it may be that the evolution of placentation evolves because it facilitates earlier maturity; the quantity of resources that are required to initiate a litter of young and their volume at initiation is smaller, allowing development to begin in a smaller, younger individual. Vitt and Blackburn (2002) have suggested that placentation evolved in the lizard genus Mabuya for this reason, but these sorts of options have yet to be considered in a theoretical model. A positive outcome of this study is that it has highlighted the additional degrees of freedom that are available to organisms that were not considered by Trexler and DeAngelis and hence has defined ways in which the model can be expanded in the future. Specifically, for matrotrophs, the assumption that terminal offspring size is the same should be changed to reflect the initial reduction in offspring mass in response to low food conditions, as shown in *P. prolifica*. Additionally, Trexler and DeAngelis assume that lecithotrophs draw lipid stores down to nearly zero before each reproductive bout. However, data from *P. monacha* indicate that lipid stores were maintained at an expense to reproduction. Finally, Trexler and DeAngelis did not include superfetation in their model. If all of these factors were incorporated in their simulations, they may change the relative advantage of the lecithotrophic strategy over the matrotrophic strategy in low food conditions.

While our goal is to address the general phenomena of matrotrophy and lecithotrophy, our study instead considers a single pair of species that differ in this fashion. It thus does not represent an absolute explanation of the differences between the two modes of maternal provisioning. The virtue of doing this kind of research in the genus *Poeciliopsis* is that it harbors three independent origins of extensive matrotrophy (Reznick et al. 2002). There is a second cluster of related species that also contains sister species that either do or do not have matrotrophy. These same experiments can thus be repeated on new species and the generality of the results can be evaluated.

REFERENCES

Bashey, F. (2002) Causes and consequences of offspring size variation in the Trinidadian Guppy (*Poecilia reticulata*).

Blackburn, D. (1992) Convergent evolution of viviparity, matrotrophy, and specializations for fetal nutrition in reptile and vertebrates. *American Zoologist* **32**, 313-321.

Borowsky, R. & Kallman, K. D. (1976) Patterns of mating in natural populations of *Xiphophorus* (Pisces:Poeciliidae). I: X. maculatus from Belize and Mexico. *Evolution* **30**, 693-706.

Charlesworth, B. *Evolution in age-structured populations* (Cambridge University Press, Cambridge, 1994).

Charnov, E. L., Downhower, J. F. & Brown, L. P. (1995) Optimal offspring sizes in small litters. *Evolutionary Ecology* **9**, 57-63.

Chen, J., & Caswell-Chen, E. P. (2004). Facultative vivipary is a life-history trait in *Caenhabditis elegans. Journal of Nematology* **36,** 107-13.

Constanz, G. D. in *Ecology and evolution of livebearing fishes* (eds Meffe, G. K. & Snelson, F. F.) 33-50 (Prentice-Hall, Englewood Cliffs, NJ, 1989).

Diamond, J. M. (1987) News and views: Causes of death before birth. *Nature* **329**, 487-488.

Einum, S. & Fleming, I. A. (2004) Environmental unpredictability and offspring size: conservative versus diversified bet-hedging. *Evolutionary Ecology Research* **6**, 443-455.

Farley, R. D. (1998) Matrotrophic adaptations and early stages of embryogenesis in the desert scorpion Paruroctonus mesaensis (Vaejovidae). *Journal of Morphology* **237**, 187-211.

Ferguson, G. W. & Fox, S. F. (1984) Annual variation of survival advantage of large juvenile side-blotched lizards, *Uta stansburiana*: its causes and evolutionary significance. *Evolution* **38**, 342-349.

Gliwicz, Z. M. & Guisande, C. (1992) Family planning in Daphnia: resistance to starvation in offspring born to mothers grown at different food levels. *Oecologia* **91**, 463-467.

Hassall, M., Walters, R., Telfer, M. & Hassall, M. R. J. (2006) Why does a grasshopper have fewer, larger, offspring at its rage limits? *Journal of Evolutionary Biology* **19**, 267.

Hodges, W. L. (2004) Evolution of viviparity in horned lizards (Phrynosoma): testing the cold climate hypothesis. *Journal of Evolutionary Biology* **17**, 1230-1237.

Hutchings, J. A. (1991) Fitness consequences of variation in egg size and food abundance in brook trout *Salvelinus fontinalis*. *Evolution* **45**, 1162-1168.

Jerez, A., & Ramirez-Pinilla, M. P. (2001). The allantoplacenta of *Mabuya mabouya* (Sauria, Scincidae). *Journal of Morphology* **249,** 132-46.

Kozlowski, J. & Stearns, S. C. (1989) Hypotheses for the production of excess zygotes: models of bet-hedging and selective abortion. *Evolution* **43**, 1369-1377.

Lalonde, R. G. (1991) Optimal offspring provisioning when resources are not predictable. *The American Naturalist* **138**, 680-686.

Lalonde, R. G. & Roitberg, B. D. (1989) Resource limitation and offspring size and number trade-offs in *Cirsium arvense* (Asteraceae). *American Journal of Botany* **76**, 1107-1113.

Laurie, W. A. & Brown, D. (1990) Population biology of marine iguanas (*Amblyrhynchus cristatus*). II. Changes in anual survival rates and the effects of size, sex, age, and fecundity in a population crash. *Journal of Animal Ecology* **59**, 529-544.

Lloyd, D. G. (1980) Sexual strategies in plants. I. An hypothesis of serial adjustment of maternal investment during one reproductive session. *New Phytologist* **86**, 69-79.

Losos, J. B., Jackman, T. R., Larson, A., de Queiroz, K. & Rodriguez-Schettino, L. (1998) Contingency and determinism in replicated adaptive radiations of island lizards. *Science* **279**, 2115-2118.

Manzur, A., Goldsman, M. P., Stone, S. C., Frederick, J. L., Balmaceda, J. P. & Asch, R. H. (1995) Outcome of triplet pregnancies after assisted reproductive techniques - how frequent are vanishing embryos. *Fertility and Sterility* **63**, 252-257.

Marsh-Matthews, E. & Deaton, R. (2006) Resources and offspring provisioning: A test of the
Trexler-DeAngelis model for matrotrophy evolution. *Ecology* **87**, 3014-3020.

McGinley, M. A. & Charnov, E. L. (1988) Multiple resources and the optimal balance between size and number of offspring. *Evolutionary Ecology* **2**, 77-84.

McGinley, M. A., Temmer, D. H. & Geber, M. A. (1987) Parental investment in offspring in a variable environment. *The American Naturalist* **130**, 370-398.

Meffe, G. K. & Vrijenhoek, R. (1981) Starvation stress and intraovarian cannibalism in livebearers (Atheriniformes: Poeciliidae). *Copeia* 702-705.

Parichy, D. M. & Kaplan, R. H. (1992) Maternal effects on offspring growth and development depend on environmental quality in the frog *Bombina orientalis*. *Oecologia* **91**, 579-586.

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

Qualls, C. P. (1997) The effects of reproductive mode and climate on reproductive success in the Australian lizard *Lerista bougainvillii*. *Journal of Herpetology* **31**, 60-65.

Reznick , D., Callahan, H. & Llauredo, R. (1996) Maternal effects on offspring quality on Poeciliid fishes. *American Zoologist* **36**, 147-156.

Reznick, D., Mateos, M. & Springer, M. (2002) Independent origins and rapid evolution of the placenta in the fish genus *Poeciliopsis*. *Science* **298**, 1018-1020.

Reznick, D. & Yang, A. (1993) The influence of fluctuation resources on life-history: patterns of allocation and plasticity in female guppies. *Ecology* **74**, 2011-2019.

Roff, D. *The Evolution of Life Histories* (Routledge, Chapman, and Hall, New York, 1992).

Shine, R. (1980) "Costs" of reproduction in reptiles. *Oecologia* **46**, 92-100.

Shine, R. (1983) Reptilian Viviparity in cold climates: testing the assumptions of an evolutionary hypothesis. *Oecologia* **57**, 397-405.

Shine, R. (2004) Does viviparity evolve in cold climate reptiles because pregnant females maintain stable (not high) body temperatures? *Evolution* **58**, 1809-1818.

Sibly, R. M. & Calow, P. (1983) An integrated approach to life-cycle evolution using selective landscapes. *Journal of Theoretical Biology* **102**, 527-547.

Smith, C. C. & Fretwell, S. D. (1974) The optimal balance between size and number of offspring. *The American Naturalist* **108**, 499-506.

Stearns, S. C. in *The evolution of sex and its consequences* (ed Stearns, S. C.) (Birkhauser, Basal, Switzerland, 1987).

Stearns, S. C. *The Evolution of Life Histories* (Oxford University Press, New York, 1992).
Thibault, R. & Schultz, R. J. (1978) Reproductive adaptations of viviparous fishes (Cyprinodontiformes: Poeciliidae). *Evolution* **32:2**, 320-333.

Tinkle, D. (1969) The concept of reproductive effort and its relation to the evolution of life histories of lizards. *American Naturalist* **103**, 501-516.

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

Trexler, J. & DeAngelis, D. (2003) Resource allocation in offspring provisioning: an evaluation of the conditions favoring the evolution of matrotrophy. *American Naturalist* **162**, 574-585 .

Turner, C. L. (1940) Pseudoamnion, pseudochorion, and follicular pseudoplacenta in poeciliid fishes. *Journal of Morphology* **67**, 59-89.

Turner, C. L. (1947) Viviparity in teleost fishes. *The Scientific Monthly* **65**, 508-518.

Turner, J. S. & Snelson, F. F. J. (1984) Population structure, reproduction, and laboratory behaviour of the introduced Belonesox belizanus (Poeciliidae) in Flordia. *Environmental Biology of Fishes* **10**, 89-100.

Vitt, L. J. & Blackburn, D. G. (2002) Reproduction in the lizard *Mabuya heathi* (Scincidae): a commentary on viviparity in new world *Mabuya*. *Canadian Journal of Zoology* **61**, 2798-2806.

Winge, O. (1937) Succession of broods in *Lebistes*. *Nature* **140**, 467.

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

Wourms, J. P., Grove, B. D. & Lombardi, J. (1988) Maternal-embryonic relationship in viviparous fishes. *Fish Physiology* **11B**, 2-133

Dependent Variable	*P. prolifica*		*P. monacha*	
	High	***Low***	***High***	***Low***
Female Mass (g)				
Before Treatment	0.499 (0.023) 22	0.504 (0.023) 22	0.929 (0.031) 16	0.940 (0.024) 19
After Treatment	0.554 (0.025)	0.420 (0.017)	0.935 (0.025)	0.731 (0.017)
Offspring Number				
Pre	12.00 (1.26) 22	11.86 (0.68) 22	9.88 (0.93) 16	10.42 (0.98) 19
Post 1	8.18 (0.90)	8.41 (0.56)	7.63 (1.08)	8.42 (1.28)
Post 2	8.95 (0.96)	8.00 (0.82)	7.94 (1.98)	8.79 (2.02)
Offspring Mass (g)				
Pre	$8.11e^{-4}$ ($3.0e^{-5}$) 21	$8.93e^{-4}$ ($3.9e^{-5}$) 22	$1.834e^{-3}$ ($6.4e^{-5}$) 13	$1.694e^{-3}$ ($6.2e^{-5}$) 19
Post 1	$8.64e^{-4}$ ($3.6e^{-5}$)	$8.00e^{-4}$ ($2.3e^{-5}$)	$1.803e^{-3}$ ($6.4e^{-5}$)	$1.598e^{-3}$ ($4.3e^{-5}$)
Post 2	$9.05e^{-4}$ ($4.2e^{-5}$)	$7.70e^{-4}$ ($4.4e^{-5}$)	$1.786e^{-3}$ ($4.6e^{-5}$)	$1.577e^{-3}$ ($6.6e^{-5}$)
Offspring Fat Content (%)				
Pre	9.39 (1.09) 20	10.92 (2.12) 19	18.17 (1.64) 12	17.53 (1.26) 15
Post 1	8.47 (2.25)	7.48 (0.72)	14.83 (1.57)	14.14 (1.74)
Post 2	12.32 (2.28)	7.98 (0.87)	11.78 (1.47)	11.84 (1.48)
Change in Interbrood Interval	0.43 (0.62) 21	1.55 (0.76) 22	1.13 (1.53) 13	1.17 (0.94) 17
Female % Fat†	17.35 (1.11) 18	10.09 (1.05) 16	5.00 (1.50) 13	2.82 (0.65) 13
RA†	0.11 (0.01) 18	0.11 (0.01) 17	0.27 (0.01) 13	0.17 (0.02) 13
Superfetation†	3.89 (0.31) 18	3.88 (0.31) 17	2.69 (0.17) 13	1.84 (0.15) 13
Total # Developing Young†	18.83 (3.61) 18	12.76 (1.96) 17	16.15 (1.88) 13	7.92 (1.27) 13

† Represents dissection data at the end of the 30 day treatments.

Table 1.1. Summary of means, standard errors (in parentheses), and sample sizes for dependent variables. Pre, Post 1, and Post 2 represent 15 days prior to treatment, days 1-15 of treatment, and days 16-30 or treatment, respectively. For repeated measures tests, sample sizes are listed on the first line only.

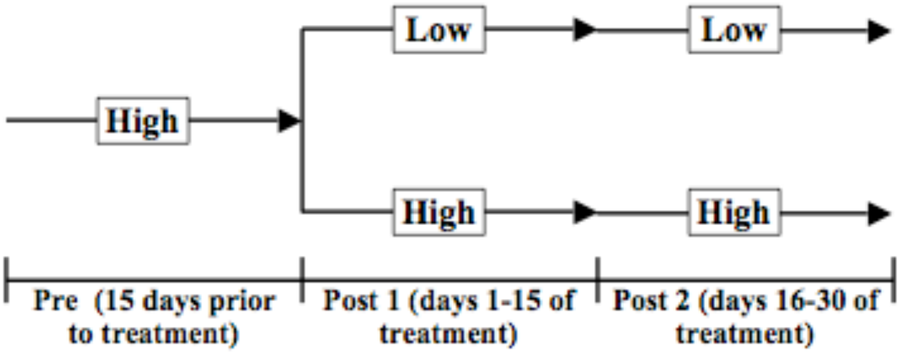
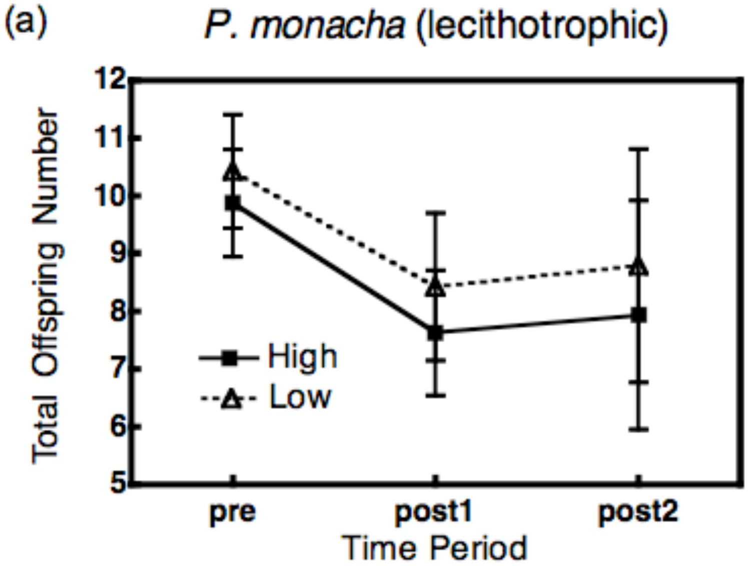


Figure 1.1. Schematic of experimental design. For each species, 15 days of data were collected before the experimental treatments began. Treatments began when approximately half of each species was switched to low food, and lasted 30 days, or approximately one gestation period. Thus, offspring born within the treatment period were initiated before the treatment began, allowing us to see the effect of food level on already developing offspring. Analyses divide data into “pre,” “post1,” and “post2” time periods. A subset of females was allowed to remain in the experiment for an additional 30 days (not shown in figure).


(a) *P. monacha* (lecithotrophic)

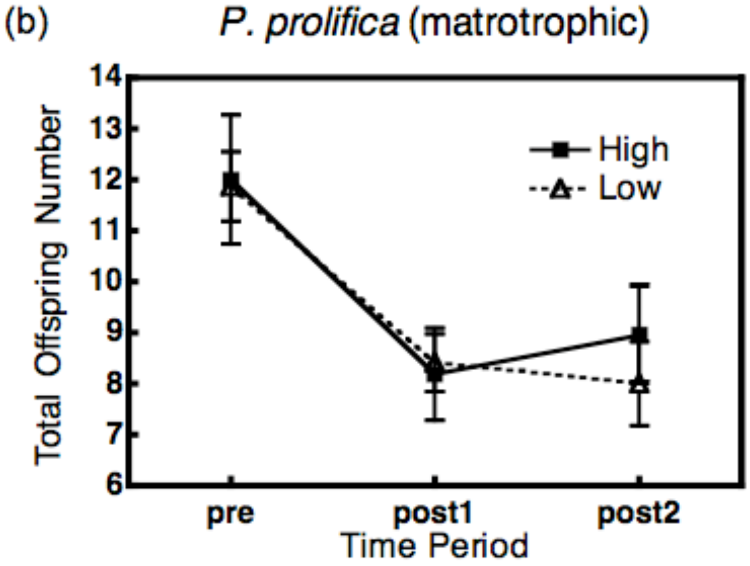

(b) *P. prolifica* (matrotrophic)

Figure 1.2. Offspring number over time in high and low food (a) *P. monacha* and (b) *P. prolifica.* Times marked 'pre' represent the 15 days prior to treatment start. 'Post1' represents days 1-15 of treatment, and 'post2' represents days 16-30 of treatment. No significant differences were found between food groups in either species.

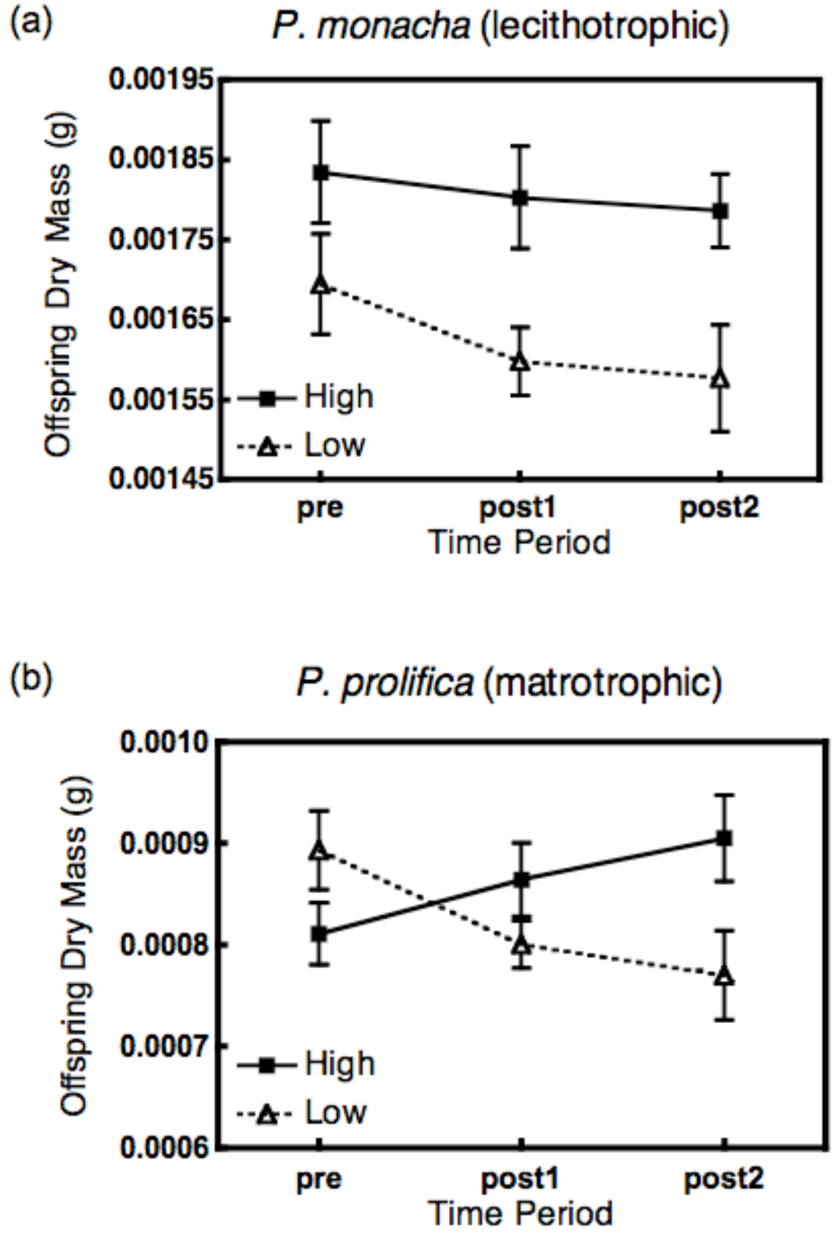


Figure 1.3. Offspring dry weight over time. (a) In *P. monacha* groups differed for unknown reasons before food treatments began, but there were no significant differences in response to food level between treatment groups. (b) High and low food groups of *P. prolifica* differed significantly in offspring dry mass over time.

CHAPTER 2:

IS MOM IN CHARGE? IMPLICATIONS OF RESOURCE PROVISIONING ON THE EVOLUTION OF THE PLACENTA.

SUMMARY: Trexler and DeAngelis presented the first mathematical model for the evolution of the placenta. The model predicts that placentas will evolve in consistent, high resource environments. We previously used closely related placental and non-placental species in the northern clade of *Poeciliopsis* to test an assumption imperative to the model, that placental species can abort a subset of developing offspring in low food conditions. Without this ability, the range of resource conditions in which a placental species can out-compete a non-placental species is extremely narrow. Our previous results show no evidence for abortion due to food level. Instead, placental species appear to be tethered to a brood once it is initiated and sacrifice body condition to maintain reproduction when resources are restricted. However, an alternative explanation for the northern clade results is that the pattern of resource allocation is a function of other life history correlates of placentation, rather than placentation alone. To distinguish between these two alternatives, we perform a similar experiment on the southern clade of the genus, which has the opposite relationship between life history traits and placentation as seen in the northern clade. Our results mirror those from the northern clade, indicating that reproductive mode, rather than life history, dictates the pattern of resource allocation. This further adds to the difficulties of explaining the evolution of the placenta within the constraints of the of the Trexler-DeAngelis model, and suggests the answer to its evolution may be found elsewhere.

INTRODUCTION

Mossman (Mossman 1937) defined placental viviparity as “any intimate apposition or fusion of the fetal organs to the maternal … tissues for physiological exchange.” Mossman also pointed out that immense structural diversity of the placenta exists across mammalian taxa. In the early part of the 19th century, this diversity was “considered as the means employed by nature to prevent the whole system respecting animals from being thrown into confusion, by preventing any two different genera from breeding together (Home 1822).” Today we know this complex union of mother and offspring performs the functions of all the major organ systems, including respiration, processing of nutrients, and waste disposal (Faber & Thornburg 1983). In the mammalian placenta, which has been has been described in more detail than any other, the placenta suppresses immunological interactions which could otherwise be fatal to a developing embryo. Additionally, zygote derived placental cells secrete hormones which affect maternal metabolism, modify maternal arterial vasculature, and maintain the uterus in the correct physiological condition for the zygote (Georgiades et al. 2002, Haig 1993). In mammals, there are over 400 genes identified that demonstrate high placental specificity in expression (Knox & Baker 2008).

Placental viviparity is a specific subset of the more general phenomenon of matrotrophy (mother-feeding); placentotrophic females have a reduced egg size at fertilization and subsequently allocate nutrients throughout gestation. Although commonly thought of as a mammalian trait, placental matrotrophy has evolved independently across a range of taxa, including reptiles, fish, and plants (Jerez &

Ramirez-Pinilla 2001, Lloyd 1980, Turner 1947, Wourms et al. 1988). Despite its frequent occurrence in nature and apparently repeated evolution, there is no general explanation for the factors that select for it. Two divergent schools of thought exist on what forces may be driving the evolution of the placenta; adaptive hypotheses and parent offspring conflict that manifests itself in utero. Adaptive hypotheses assume that the placenta evolves in response to some external ecological selection pressure in the environment (Thibault & Schultz 1978, Trexler & DeAngelis 2003). Conflict hypotheses view the placenta as the product of antagonistic coevolution between the mother and offspring (Crespi & Semeniuk 2004, Haig 1993, Haig 1996). In an adaptive scenario, the placenta is an organ that increases the mother's lifetime fitness, whereas in the conflict hypotheses, it has been likened to a tug-of-war (Haig 1993) where the selection favors offspring that elicit more resources than is in the best interest in the mother to give them, and counter-selection favors mothers that limit access to resources. Adaptive hypotheses assume that the mother is 'in charge' of resource allocation, whereas in conflict hypotheses, there is an ongoing struggle between mother and offspring over the allocation of resources. These hypotheses are not necessarily mutually exclusive.

Adaptive hypotheses generally fall into one of three categories: Locomotor costs, life history facilitation, or resource availability hypotheses. Locomotor hypotheses are based on the assumption that morphological and/or physiological changes during pregnancy detrimentally affect escape responses or sustained swimming abilities, and that placental matrotrophy can alleviate some of these costs because it confers a decrease in the mean reproductive allocation over the course of pregnancy (Thibault & Schultz

1978). Several studies have shown that females with larger reproductive allocations have decreased locomotor capacity and increased susceptibility to predators (Ghalambor et al. 2004, Plaut 2002, Walker et al. 2005). The life history facilitation hypothesis suggests that the placenta evolved because it facilitated the evolution of some other life history trait that was under positive selection. This hypothesis has little support, as the correlation between placentation and other life history traits is inconsistent across taxa (Reznick et al. 2007). Resource availability hypotheses are based upon the timing of resource allocation. Because placental species start with a small egg and allocate nutrients throughout the development period, they spread reproductive investment over time. A consequence of protracted resource allocation is that placental organisms need a relatively low but steady influx of resources to be able to allocate nutrients to developing embryos. Non-placental species make their investment prior to fertilization, meaning that peak demand is very high, but after the initial investment, embryos are buffered from environmental instability. This difference in timing could potentially affect which reproductive strategy has an advantage under a given resource level and temporal pattern of resource availability (Trexler & DeAngelis 2003).

Alternatively, other authors postulate the placenta arose from antagonistic coevolution due to parent-offspring conflict in utero (Crespi & Semeniuk 2004, Haig 1993, Trivers 1974, Zeh & Zeh 1996), sometimes termed the viviparity conflict hypothesis. Because mother and offspring are not genetically identical, natural selection acting on the mother may oppose that acting on the offspring (Haig 1993). A female’s fitness is highest when all of her offspring survive and reproduce successfully. However, if an offspring gains a fitness benefit by eliciting more resources from the mother, and this benefit outweighs the inclusive fitness costs that may be incurred via its siblings or mother, then any trait that allows an offspring to gain more resources will be beneficial for it. This conflict is magnified when a female mates with multiple males, because the coefficient of relatedness between siblings is reduced, reducing inclusive fitness costs. Thus, parent-offspring conflict is expected to result in a red-queen process of adaptation and counter adaptation, where the offspring evolves traits that allow it to elicit more resources from the mother than is in her best interest, and the mother is counters with an adaptation that forms a barrier to overexploitation by the offspring (Crespi & Semeniuk 2004, Haig 1993, Trivers 1974). Viviparity provides a potential arena for this conflict to take place during gestation (Trivers 1974). The intimate connection between mother and offspring via the placenta has been likened to a battlefield, where the maternal tissue can be seen as a defender against an invading trophoblast, which is of embryonic origin (Haig 1993). Modifications of the maternal arterial vasculature and hormone secretion by the fetal placental tissue may be an offspring's attempt to manipulate the mother into allocating more resources than is in her best interest. Resultantly, an arms race may occur where offspring are selected to manipulate the mother, and females counter this with an adaptation to resist such manipulation, the product being a placenta of growing complexity over many generations.

Previous Experimental Work

In a previous study (Banet & Reznick 2008) we experimentally examined several features of Trexler and DeAngelis' adaptive hypothesis for the evolution of matrotrophy. Trexler and DeAngelis used a combination of analytical and simulation techniques to examine the ecological conditions under which a matrotroph would have greater reproductive success than a lecithotroph (Trexler & DeAngelis 2003). The model assumes that individuals with either form of maternal provisioning attain maturity with the same quantity of resources and that there is a tradeoff between the number of eggs that are initially fertilized and the size of the eggs. Because matrotrophs start with small eggs and then provision them during development, they can fertilize and initiate the development of a larger number of eggs than lecithotrophs. Given enough resources to provision the embryos during gestation, the matrotroph thus has the potential to have a larger clutch size. If resources are low or unpredictable, the ability for a matrotroph to abort a subset of the brood is crucial. In the model, if they cannot abort offspring, then the matrotroph risks spreading resources too thin and losing all offspring in a brood.

In the previous study, we compared closely related lecithotrophic and matrotrophic species from the northern clade of *Poeciliopsis*, which includes one independent origin of matrotrophy (Banet & Reznick 2008). We compared matrotrophic *Poeciliopsis prolifica* to a closely related lecithotrophic species, *Poeciliopsis monacha,* in order to test the assumption from the Trexler-DeAngelis model that matrotrophic species abort offspring in low food conditions (Trexler & DeAngelis 2003). We found no evidence for abortion. Instead, we found that in low food conditions, matrotrophic *P. prolifica* sacrificed lipid reserves in order to maintain developing embryos. Lecithotrophic *P. monacha* instead sacrificed future reproduction in order to maintain lipid reserves (Banet & Reznick 2008). Low food *P. prolifica* females also produced smaller offspring. *Poeciliopsis monacha* females did not alter offspring size in response to food availability. Research on other organisms has shown that smaller offspring suffer a loss of fitness when food is scarce because of competition from larger offspring (Einum & Fleming 2004, Ferguson & Fox 1984, Gliwicz & Guisande 1992, Hassall et al. 2006, Hutchings 1991, Parichy & Kaplan 1992). The response of *P. prolifica* to low food thus appears to be maladaptive. It seems rather than aborting offspring in low food conditions, placental species are committed to the entire brood once it is initiated, even to the point of being unable to fully provision them all. This result suggests that the mother is not in full charge of resource allocation and instead lends some support to conflict hypotheses for the evolution of the placenta. If the mother were fully in control of resource partitioning, she would be expected to produce fewer, larger offspring in low food conditions (Einum & Fleming 2004, Ferguson & Fox 1984, Gliwicz & Guisande 1992, Hassall et al. 2006, Hutchings 1991, Parichy & Kaplan 1992) as is seen in several non-placental species of Poeciliidae (Reznick et al. 1996, Reznick & Yang 1993). Under the Trexler-DeAngelis model, the absence of the ability to abort offspring severely narrows the conditions which matrotrophy would be favored over lecithotrophy.

The presence of the placenta may be essential to our previous results, but there is a potential alternative explanation. In life history theory, there is a predicted association between the evolution of early maturity, a higher rate of investment in reproduction and a preferential investment in current reproduction, as opposed to aspects of "residual reproductive value", like somatic growth and maintenance (Roff 1992, Stearns 1992, Williams 1966). All of these traits characterize the life history of *P. prolifica* in comparison to *P. monacha*. *Poeciliopsis prolifica* matures at an earlier age, has a higher rate of investment in reproduction early in life, and produces more and smaller offspring than *P. monacha* (Thibault & Schultz 1978). It is thus plausible that the pattern of resource allocation we see in *P. prolifica* is a consequence of the evolution of this life history rather than a direct result of the presence or absence of the placenta.

Here we test the generality of the northern clade results by performing the same experiment on two species from the southern clade of *Poeciliopsis*, which includes a second independent origin of the placenta (Reznick et al. 2002). The southern clade allows us to distinguish between the two alternatives presented above because these species have a very different association between the placenta and the remainder of the life history. In contrast to the northern clade, southern clade placental species have delayed maturity, produce fewer, larger offspring and allocate resources to reproduction at a lower rate than lecithotrophic species (Bassar et al, in prep). If the pattern of resource commitment seen in the northern clade is due to features of the life history (age at maturity, number and size of offspring), then the present study will show that lecithotrophic species respond to food reduction by sustaining the rate of offspring production at the expense of somatic reserves. If instead it is the presence of the placenta that dictates how females respond to changes in food availability, then the results of this experiment should mirror what we saw in the northern clade. This result would further add to the difficulties of explaining the evolution of the placenta within the constraints of the Trexler-DeAngelis model, and suggests the answer to its evolution may be found elsewhere.

METHODS

Poeciliopsis gracilis and *Poeciliopsis turneri* are closely related species found in the southern clade of *Poeciliopsis* (Mateos et al. 2002). Both species are livebearers with internal fertilization. *Poeciliopsis gracilis* is non-placental, with its embryos losing 30-40% of dry mass over development. *Poeciliopsis turneri* has extensive placentation, with embryos showing a 30 to 40-fold increase in dry mass over the course of development. Both species can store sperm (Banet personal observation), which allowed us to keep females in isolation for the course of the experiment. Gestation time is estimated to be approximately 30 days based on data from closely related species (Pires and Reznick, unpublished data). A key component for this experiment is the presence of superfetation in both species. Superfetation, the ability to carry multiple broods in different stages of development, means that at any given time a reproducing female will have a 30-day supply of babies developing inside her in an assembly-line fashion and can give birth every few days.

We followed methods in Banet and Reznick (2008) with minor modifications. A factorial design with food treatment (high/low) and reproductive mode (placental/non-placental) was used. All fish were kept on a 12/12 hour light/dark cycle. Females were reared in group aquaria with males. Once actively reproducing, each female was housed individually in 8-liter aquaria. Tanks were checked daily for babies, which were collected and preserved. Data were recorded for 15 days with all females on high food rations. After 15 days, half of each species was randomly switched to low food. This period lasted 30 days, the estimated gestation time. Offspring born during the 30-day experiment were thus initiated before treatments began. A reduction in the rate of offspring production during this 30 day interval in the low food group as compared to the high food group is indicative of abortion due to food level. Because both species have superfetation, we can see how embryos at different stages of development are affected by a reduction in food level. Offspring born early in the 30-day treatment period would have been in advanced stages of development when the treatment began, while those born late in the 30-day treatment period would have been at early stages of development. At the end of the 30 days, females were sacrificed and preserved for dissection. See figure 2.1 for a schematic of the design.

The *P. gracilis* were derived from a 2003 collection from the Rio Jones in Guatemala. *Poeciliopsis turneri* came from Rio Purificacion in Mexico and were derived from a collection made in 2004. Fish were fed liverpaste in the morning and newly hatched *Artemia nauplii* (brine shrimp) in the afternoon. High food levels were just under ad libitum levels for each species. Low food levels were one-fifth of the high food level. For *P. gracilis*, high food levels were 125 μL per meal, and low food levels were 25 μL per meal. *Poeciliopsis turneri* high food females were fed 200 μL per meal, and low food levels were 40 μL per meal. Our *P. turneri* stocks took longer to mature than predicted, causing temporal difference in the start of the experiment for each species. They were also larger and hence demanded a larger ration to sustain a rate of growth similar to *P. gracilis* (Mean female mass at start of experiment: *P. gracilis* 1.072g, *P. turneri* 1.318g). Data for *P. gracilis* was collected from October 14, 2006 until November 27, 2006. Data for *P. turneri* was collected from April 26, 2007 until June 8, 2007. When *P. turneri* did begin reproducing, litter size was usually one, which would give us little power to detect a decrease in litter size. In order to increase litter size, we supplemented high food levels for *P. turneri* prior to the 30 day experiment with adult brine shrimp each evening.

Preserved females were dissected and processed following methods in Reznick and Yang (Reznick & Yang 1993). During the experiment, dependent variables quantified were offspring dry mass, offspring fat content, and female mass. After the experiment, females were dissected to quantify reproductive allocation, degree of superfetation, female dry mass, female fat content, number of developing young, and their stage of development. We used a repeated measures ANOVA to analyze livebirths of offspring, offspring dry mass and offspring fat content, with food level as the independent variable. The repeated measures were the 15 days prior to the treatments, days 1-15 of the treatment, and days 16 -30 of the treatment. Size was not needed as a covariate in these analyses because the dependent variables were not correlated with size. A one-way ANOVA was used to analyze changes in reproductive allocation, degree of superfetation, female dry mass, female fat content, number of developing young, *P. gracilis* mean embryo dry mass, and *P. turneri* ovary dry mass. Stage of development was correlated with *P. turneri* mean embryo dry mass, and female dry mass was correlated with *P. gracilis* ovary dry mass. A one-way ANCOVA was thus used for these analyses. Thirteen *P. gracilis* and six *P. turneri* females were excluded from analyses of offspring dry mass and fat content because they did not give birth in one or more of the time intervals. Eight *P. gracilis* and one *P. turneri* female were excluded from mean embryo dry mass analysis because they did not have any developing embryos when dissected. Ten *P. gracilis* (five from each treatment group) were kept alive for use in another study, thus we do not have dissection data for those individuals at the end of the experiment. These deletions are noted in the degrees of freedom, reported below.

RESULTS

Female Mass: The average wet mass of females in the high and low food treatments was not different at the start of the experiment. At the end of the experiment, high food *P. gracilis* females were 124% of their mass at the start of the food treatments. Low food *P. gracilis* females weighed 92% of their initial mass. In placental *P. turneri*, high food females were 122% and low food females were 94% of their initial mass (Table 2.1; Fig. 2.2: Repeated measures ANOVA: *P. gracilis*: $F_{1,38} = 78.551$ $p < 0.001$; *P. turneri*: $F_{1,44} = 50.994$ $p < 0.001$). This gives us confidence that experimental food treatments were sufficiently different to invoke a response.

Offspring Number: Neither species showed a significant difference in the total number of offspring born between high and low treatment groups over the course of the experiment. (Table 2.1; Fig. 2.3: Repeated measures ANOVA: *P. gracilis*: $F_{2,47} = 1.963$ $p = 0.152$; *P. turneri*: $F_{2,43} = 0.853$ $p = 0.433$) In matrotrophic *P. turneri*, both treatment groups showed an increase in fecundity over the course of the experiment. This is likely because we increased food rations for all *P. turneri* approximately 40 days before the experiment began in order to stimulate larger brood sizes during the experiment. Broods born early in the experiment would be initiated shortly after the increase occurred while those born late in the experiment would have been initiated four or more weeks after this pre-experiment increase in ration level. Since there was no difference in fecundity *between* the high and low food groups, the results imply that no abortion of developing offspring occurred in response to the decrease in food level.

Offspring Dry Mass: Neither species showed a significant difference in the dry mass of offspring between high and low treatment groups over the course of the experiment. Both species do show a non-significant trend reminiscent of the northern clade results, with high food females showing increased offspring mass and low food females showing decreased offspring mass over the course of the experiment (Table 2.1; Fig. 2.4: Repeated measures ANOVA: P. gracilis: $F_{2,21} = 2.392$ $p = 0.116$; P. turneri: $F_{2,33} = 2.014$ $p = 0.150$).

Offspring Fat Content: Non-placental *P. gracilis* showed no difference in offspring composition between treatment groups (Table 2.1; Fig. 2.5b: Repeated measures ANOVA: $F_{2,21} = 0.556$ $p = 0.582$). In placental *P. turneri* the percent fat of offspring decreased in the low food over the course of the experiment, while in the high food group, percent fat increased (Table 2.1; Fig. 2.5d: Repeated measures ANOVA: $F_{2,33}$ = 4.528 p = 0.018).

Female Lipids: At the end of the experiment, non-placental *P. gracilis* showed no significant difference in the percentage of fat in females between treatment groups (Table 2.1; Fig. 2.6b: $F_{1,38}$ = 1.880 p = 0.178). In placental *P. turneri,* low food females had significantly lower fat levels than high food females (Table 2.1; Fig. 2.6b: $F_{1.44}$ = 10.557 p = 0.002)

Reproductive Allocation: Low food *P. gracilis* (non-placental) showed a significantly lower reproductive allocation (reproductive dry mass/total dry mass) than high food females at the end of the experiment (Table 2.1; Fig. 2.6d: $F_{1,38}$ = 27.442 $p < 0.001$). No significant difference was found between treatment groups in placental *P. turneri* (Table 2.1; Fig. 2.6d: $F_{1,44}$ = 3.429 p = 0.071).

Superfetation: In *P. gracilis* (non-placental), there was no difference in the number of developing broods found in high and low treatment groups at the end of the experiment (Table 2.1: $F_{1,38}$ = 0.162 p = 0.690). Low food *P. turneri* females (placental) had fewer developing broods than high food females (Table 2.1: $F_{1,44}$ = 4.435 $p < 0.041$).

We estimated a range of variables based on the dissection of the females after the end of the 30 day experiment. These variables provide some projection into the future of the likely consequences of the experimental treatments because they characterize some aspects of future reproduction.

Number of Developing Young: Non-placental *P. gracilis* showed no difference in the total number of developing young between in high and low treatment groups at the end of the experiment (Table 2.1: $F_{1,38} = 0.037$ $p = 0.848$). Low food *P. turneri* females (placental) had fewer developing young than high food females (Table 2.1: $F_{1,44} = 12.028$ $p = 0.001$).

DISCUSSION

The strength of the *Poeciliopsis* study system is that there are multiple, independent origins of a complex trait. The genus contains two sets of closely related species that do or do not have a placenta, which provides enhanced opportunities to ask how and why the trait evolved. Previously we evaluated a key assumption of the Trexler-DeAngelis model, that matrotrophic species abort offspring in response to low food availability. To do this, we used a species with an independent evolutionary origin of placental matrotrophy and a closely related non-placental species in the northern clade of the genus *Poeciliopsis*. We found no evidence for abortion in placental species due to low food availability. Additionally, we found that that the placental species sacrificed body condition to maintain reproduction, and the non-placental species maintained body condition at the expense of reproduction (Banet & Reznick 2008).

Here we repeat the study on an independent origin of placentation in the southern clade of *Poeciliopsis* in order to test the generality of these results. In comparison to the northern clade, the southern clade shows an inverse relationship between the presence of the placenta and the suite of classic life history traits (Thibault & Schultz 1978, Bassar et al., in prep). In contrast to the northern clade, southern clade placental species have delayed maturity, produce fewer, larger offspring and allocate resources to reproduction at a lower rate than non-placental species. Repeating the experiment thus allows us to distinguish whether the results from the previous study are due to the presence or absence of the placenta, or whether it is a function of the life history of the organism.

We found that responses to reduced food availability in this study were similar to those in the northern clade (see Table 2.2 for a summary of both clades). Specifically, we found no evidence for abortion in matrotrophic *P. turneri* due to low food conditions. If abortion occurred, then the low food group would have shown a reduction in offspring number as compared to the high food group within one gestation period (*c.* 30 days). However, there were neither significant differences nor even compelling trends in offspring number between the high and low food groups in either species (Fig 2.3). We also found the same relationship between reproductive allocation and female lipids at the end of the experiment (Fig 2.6) in both experiments. Matrotrophic *P. turneri* maintained reproductive allocation in low food conditions, but showed a significant reduction in somatic fat reserves, as seen in *P. prolifica* in the earlier study. In contrast, lecithotrophic *P. gracilis* reduced reproductive allocation in low food conditions, but maintained lipid reserves, as seen in *P. monacha* in the earlier study. Interestingly, in both the northern and southern clades, the matrotrophic species shows a higher female lipid content, thus has a greater reserve to draw from in low food conditions. This is in contrast to the Trexler-DeAngelis model, where matrotrophs were leaner throughout the reproductive season than lecithotrophs. This could represent an adaptation to low food conditions, but further controlled study is needed to examine the biological significance of this difference. The allocation of resources to offspring, measured by size and fat content, declined in placental species *(P. prolifica* and *P. turneri)* in response to a decrease in food availability after fertilization. In the northern clade, this was manifested in a decrease in offspring dry mass. In the present study on the southern clade, it was reflected as a decrease in the proportion of lipids in the offspring. Both a decrease in size and lipid proportions has been shown to have a detrimental effect on offspring survival, particularly in low resource conditions (Einum & Fleming 2004, Ferguson & Fox 1984, Gliwicz & Guisande 1992, Hassall et al. 2006, Hutchings 1991, Parichy & Kaplan 1992), so this reduction in allocation is potentially a reduction in offspring quality. Because the northern and southern clade show inverse relationships between the presence of the placenta and the suite of other life history traits, comparison of the data from the two clades indicate that the pattern of resource allocation (maintenance vs. reproduction) we see is due to the presence of the placenta, rather than being a correlate of the other life history traits.

Dissection of the females at the end of the experiment showed slightly different patterns between the northern and southern clades. In the northern clade non-placental females respond to food restriction by initiating fewer broods and reducing the total number of developing offspring. Food restriction had no significant effect on the degree of superfetation or number of developing offspring in northern clade placental species (Banet & Reznick 2008). The present study on the southern clade shows an inverse trend; non-placental *P. gracilis* shows no significant reduction in the degree of superfetation and number of developing offspring under the low food regime, while placental *P. turneri* produces fewer broods and fewer embryos. It should be noted however that while *P. prolifica* and *P. gracilis* show no significant difference in the degree of superfetation or the total number of developing young, the trend in all four species is the same; low food females have lower fecundity (as projected by the number developing embryos) than high food females in broods initiated after the food treatments began.

These results tell us two important things. Firstly, they suggest incongruence between the Trexler-DeAngelis model and the biology of matrotrophic species. Placental species are tethered to developing offspring once fertilization occurs. In the face of severe food restriction, they drain somatic reserves to sustain developing young, but also fall short in sustaining their young and produce offspring that are likely to be of lower quality. This same reduction of offspring quality in response to food restriction was observed in an earlier study of *Heterandria formosa*, another Poeciliid that has independently evolved a placenta (Reznick et al. 1996). Without the ability to abort, the conditions in which a matrotroph will have greater reproductive success than a lecithotroph are narrowed to only the highest resource conditions under the Trexler-DeAngelis model. The pattern of resource partitioning to reproduction versus somatic tissues further reduces the likelihood that a matrotroph could out-compete a lecithotroph in any but the highest resource levels. By highlighting the biological realities of the matrotrophic and lecithotrophic reproductive modes, this study has thus narrowed the conditions under which the Trexler-DeAngelis model can explain the evolution of matrotrophy and suggests that the true answer to the evolution of the placenta may lie elsewhere.

Secondly, these results give clues to where the explanation for the evolution of the placenta may lie. As described above, producing lighter or leaner offspring detrimentally affects offspring survival in low resource conditions. Thus, we would expect if a mother were fully in charge of partitioning resources to developing offspring, in low food conditions she would abort a subset of embryos in order to increase the viability of the remaining offspring. One may argue that the low food treatment in our experiment was not sufficiently low enough to invoke the female to abort, and if female lipids had been drawn down to a greater extent, then the some embryos would be aborted due to the female's inability to provision it. We recognize this possibility, but unless abortion occurs before the deterioration of offspring quality and female condition, the female is likely to suffer a fitness loss due to decreased offspring survival and a reduction in future reproduction. The absence of abortion *before* this deterioration occurs indicates the mother may not be fully in control of resource allocation. Offspring gain a fitness benefit by both surviving gestation and by eliciting as many resources from the mother as possible in order to increase size and viability in the low food environment it is about to enter. However, in the present study and the previous study on the northern clade, the outcome seems to be maladaptive for both mother and offspring, suggesting parent-offspring conflict in utero may be mediating this interaction.

Several other lines of evidence in Poeciliid fishes also give indirect support for the viviparity conflict hypothesis (for detailed description of predictions, see Crespi and Semeniuk 2003). One prediction is the occurrence of multiple independent origins of the placenta, which reflect a high level of rapidly occurring interspecific diversity. This is corroborated by several studies that have documented immense anatomical and functional diversity of the Poeciliid placenta (Grove & Wourms 1991, Grove & Wourms 1994, Reznick et al. 2002, Turner 1940). The viviparity conflict hypothesis also predicts a positive correlation between placental complexity and the degree of post-fertilization maternal investment. Turner (1940) found that in the genus *Poeciliopsis*, a decrease in the size of the yolk sac (indicating a reduction of maternal investment prior to fertilization), is associated with an increase in vascularity and in the number and size of microvilli in the follicular placenta. A third prediction is that genes expressed in the placenta will evolve more rapidly than their non-placental counterparts. Evidence for this comes from *insulin-like growth factor II (IGF2)*, a gene thought to have a major influence on embryonic growth and has conserved function across vertebrates. Studies in viviparous teleost fishes show that *IGF2* shows a high ratio of nonsynonomous to silent mutations at protein codon sites, suggesting strong positive selection (O'Neill et al. 2007).

Our results reveal that the biology of matrotrophic species presents inherent problems in using the Trexler-DeAngelis model to explain the evolution of the placenta, at least in its current form. Additionally, these results lend credence to a growing number of studies that point to parent-offspring conflict in utero as a potential explanation for the evolution of the placenta. However, further study is needed in order to verify conflict as a driving force in the evolution of the placenta.

REFERENCES

Banet, A. I. & Reznick, D. N. (2008). Do placental species abort offspring? Testing an assumption of the Trexler-DeAngelis model. *Functional Ecology* **22**, 323-331.

Crespi, B. & Semeniuk, C. (2004). Parent-offspring conflict in the evolution of vertebrate reproductive mode. *American Naturalist* **163**, 635-653.

Einum, S. & Fleming, I. A. (2004). Environmental unpredictability and offspring size: conservative versus diversified bet-hedging. *Evolutionary Ecology Research* **6**, 443-455.

Faber, J. J. & Thornburg, K. L. *Placental Physiology: Structure and Function of Fetomaternal Exchange* (Raven Press, New York, 1983)

Ferguson, G. W. & Fox, S. F. (1984). Annual variation of survival advantage of large juvenile side-blotched lizards, *Uta stansburiana*: its causes and evolutionary significance. *Evolution* **38**, 342-349.

Georgiades, P., Ferguson-Smith, A. C. & Burton, J. G. (2002). Comparative developmental anatomy of the murine and human definitive placentae. *Placenta* **23**, 3-19.

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

Gliwicz, Z. M. & Guisande, C. (1992). Family planning in Daphnia: resistance to starvation in offspring born to mothers grown at different food levels. *Oecologia* **91**, 463-467.

Grove, B. D. & Wourms, J. P. (1994). Follicular placenta of the viviparous fish, *Heterandria formosa*: II. Ultrastructure and development of the follicular epithelium. *Journal of Morphology* **220**, 167-184.

Grove, B. D. & Wourms, J. P. (1991). The follicular placenta of the viviparous fish, *Heterandria formosa*. I. Ultrastructure and development of the embryonic absorptive surface. *Journal of Morphology* **209**, 265-284.

Haig, D. (1993). Genetic conflicts in human pregnancy. *Quarterly Review of Biology* **68:4**, 495-532.

Haig, D. (1996). Gestational drive and the green-bearded placenta. *Evolution* **93**, 6647-6551.

Hassall, M., Walters, R., Telfer, M. & Hassall, M. R. J. (2006). Why does a grasshopper have fewer, larger, offspring at its range limits? *Journal of Evolutionary Biology* **19**, 267.

Home, E. (1822). On the placenta. *Philosophical Transactions of the Royal Society of London* **112**, 401-407.

Hutchings, J. A. (1991). Fitness consequences of variation in egg size and food abundance in brook trout *Salvelinus fontinalis*. *Evolution* **45**, 1162-1168.

Jerez, A. & Ramirez-Pinilla, M. P. (2001). The allantoplacenta of Mabuya mabouya (Sauria, Scincidae). *Journal of Morphology* **249**, 132-146.

Knox, K. & Baker, J. (2008). Genomic Evolution of the placenta using co-option and duplication and divergence. *Genome Research* **18**, 695-705.

Lloyd, D. G. (1980). Sexual strategies in plants. I. An hypothesis of serial adjustment of maternal investment during one reproductive session. *New Phytologist* **86**, 69-79.

Mateos, M., Sanjur, O. & Vrijenhoek, R. (2002). Historical biogeography of the livebearing fish genus *Poeciliopsis* (Poeciliidae: Cyprinodontiformes). *Evolution* **56**, 972-984.

Mossman, H. W. (1937). Comparative morphogenesis of the fetal membranes and accessory uterine structures. *Contributions to Embryology* **26**, 129-146.

O'Neill, M. J., Lawton, B. R., Mateos, M., Carone, D. M., Ferreri, G. C., Hrbek, T., Meredith, R., Reznick, D. & O'Neill, R. J. (2007). Ancient and continuing Darwinian selection on insulin-like growth factor II in placental fishes. *Proceedings of the National Academy of Sciences* **104**, 12404-12409.

Parichy, D. M. & Kaplan, R. H. (1992). Maternal effects on offspring growth and development depend on environmental quality in the frog *Bombina orientalis*. *Oecologia* **91**, 579-586.

Plaut, I. (2002). Does pregnancy affect swimming speed in female mosquitofish, *Gambusia affinis? Functional Ecology* **16**, 290-295.

Reznick, D., Callahan, H. & Llauredo, R. (1996). Maternal effects on offspring quality on Poeciliid fishes. *American Zoologist* **36**, 147-156.

Reznick, D., Meredith, R. & Collette, B. B. (2007). Independent evolution of complex life history adaptations in two families of fishes, live-bearing halfbeaks (Zenarchopteridae, Beloniformes) and Poeciliidae (Cyprinodontiformes). *Evolution* **61**, 2570-2583.

Reznick, D., Mateos, M. & Springer, M. (2002). Independent origins and rapid evolution of the placenta in the fish genus *Poeciliopsis*. *Science* **298**, 1018-1020.

Reznick, D. & Yang, A. (1993). The influence of fluctuation resources on life-history: patterns of allocation and plasticity in female guppies. *Ecology* **74**, 2011-2019.
Roff, D. *The Evolution of Life Histories* (Routledge, Chapman, and Hall, New York, 1992)

Stearns, S. C. *The Evolution of Life Histories* (Oxford University Press, New York, 1992)
Thibault, R. & Schultz, R. J. (1978). Reproductive adaptations of viviparous fishes (*Cyprinodontifomes: Poeciliidae)*. **32:2**, 320-333.

Trexler, J. & DeAngelis, D. (2003). Resource allocation in offspring provisioning: an evaluation of the conditions favoring the evolution of matrotrophy. *American Naturalist* **162**, 574-585.

Trivers, R. (1974). Parent-offspring conflict. *American Zoologist* **14**, 249-264.
Turner, C. L. (1940). Pseudoamnion, pseudochorion, and follicular pseudoplacenta in Poeciliid fishes. *Journal of Morphology* **67**, 59-87.

Turner, C. L. (1947). Viviparity in teleost fishes. *The Scientific Monthly* **65**, 508-518.

Walker, J. A., Ghalambor, C., Griset, O. L., McKenney, D. & Reznick, D. (2005). Do faster starts increase the probability of evading predators? *Functional Ecology* **19**, 808-815.

Williams, G. C. (1966). Natural selection, the costs of reproduction, and a refinement of Lack's Principle. *American Naturalist* **100**, 687-690.

Wourms, J. P., Grove, B. D. & Lombardi, J. (1988). Maternal-embryonic relationship in viviparous fishes. *Fish Physiology* **11B**, 2-133.

Zeh, J. & Zeh, D. (1996). The evolution of polyandry I: intragenomic conflict and genetic incompatibility. *Proceedings of the Royal Society* **263**, 1711-1717.

Dependent Variable	*P. gracilis*		*P. turneri*	
	high food	***low food***	***high food***	***low food***
Female Mass (g)				
Before Treatment	1.036 (0.048) 20	1.107 (0.047) 20	1.289 (0.046) 23	1.347 (0.007) 23
After Treatment	1.287 (0.057)	1.025 (0.034)	1.570 (0.060)	1.268 (0.061)
Offspring Number				
Pre	12.20 (0.88) 25	12.56 (0.92) 25	1.41 (0.23) 23	1.50 (0.19) 23
Post 1	9.68 (1.30)	7.120 (1.36)	1.89 (0.24)	1.63 (0.19)
Post 2	8.88 (1.44)	10.96 (1.46)	3.22 (0.26)	2.83 (0.30)
Offspring Dry Mass (g)				
Pre	1.47e^{-3} (9.4e^{-5}) 12	1.42e^{-3} (8.0e^{-5}) 12	7.41e^{-3} (5.74e^{-4}) 17	7.75e^{-3} (3.17e^{-4}) 19
Post 1	1.57e^{-3} (6.0e^{-5})	1.48e^{-3} (3.1e^{-5})	7.40e^{-3} (4.20e^{-4})	7.48e^{-3} (4.04e^{-4})
Post 2	1.56e^{-3} (7.4e^{-5})	1.37e^{-3} (7.7e^{-5})	7.75e^{-3} (3.25e^{-4})	6.84e^{-3} (4.52e^{-4})
Offspring Fat Content (%)				
Pre	17.56 (1.29) 12	17.53 (1.15) 12	26.89 (1.45) 17	30.17 (0.92) 19
Post 1	17.95 (1.10)	19.27 (1.10)	29.67 (1.26)	28.84 (0.93)
Post 2	17.56 (1.34)	18.53 (1.78)	29.66 (0.97)	26.77 (1.11)
Female % Fat†	15.01 (0.86) 20	13.29 (0.93) 20	29.24 (1.60) 23	21.31 (1.85) 23
RA†	0.21 (0.01) 20	0.13 (0.01) 20	0.07 (0.01) 23	0.08 (0.01) 23
Superfetation†	1.15 (0.20) 20	1.05 (0.15) 20	2.52 (0.15) 23	2.09(0.14) 23
Total # Developing Young†	9.70 (3.00) 20	9.05 (1.56) 20	8.96 (0.75) 23	5.82 (0.50) 23

† Represents data from dissections after the experiment.

Table 2.1. Means, standard errors (in parentheses), and sample sizes for dependent variables. Pre, post 1, and post 2 represent the 15 days prior to treatment, days 1-15 of treatment, and days 16-30 of treatment, respectively. Sample sizes are listed on the first line only for repeated measures tests.

	Northern Clade		Southern Clade	
	P. prolifica (placental)	***P. monacha*** (non-placental)	***P. turneri*** (placental)	***P. gracilis*** (non-placental)
Offspring Number	H = L	H = L	H = L	H = L
Offspring Dry Mass (g)	**H > L**	H = L	H = L	H = L
Offspring Fat Content (%)	H = L	H = L	**H > L**	H = L
Female % Fat†	**H > L**	H = L	**H > L**	H = L
RA†	H = L	**H > L**	H = L	**H > L**
Superfetation†	H = L	**H > L**	**H > L**	H = L
Total # Developing Young†	H = L	**H > L**	**H > L**	H = L

†Represents data from dissections after the experiment.

Table 2.2. Comparison of results in high (H) and low (L) food groups from the northern and southern clades. All species showed no significant difference in offspring number between high and low food groups, giving no evidence that abortion occurs in low food conditions. Placental species of both clades produced lower quality offspring in low food conditions. In the northern clade, this was manifested as a reduction in offspring mass; in the southern clade, it was a reduction in offspring lipid content. In both clades, placental species sacrificed female fat content in order to maintain reproductive allocation, while the non-placental species maintained female lipids at the expense of reproduction. In low food groups, superfetation and total number of developing young were significantly reduced in non-placental *P. monacha* of the northern clade, and placental *P. turneri* in the southern clade. However, the trend in these two variables was the same for all four species.

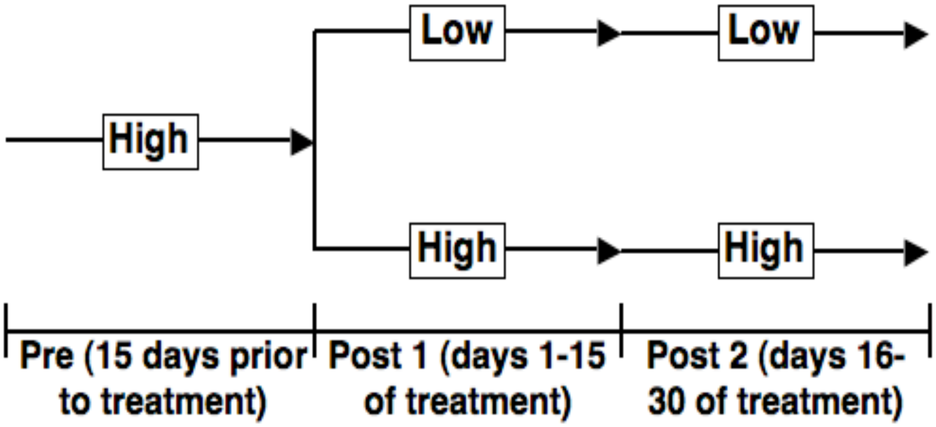
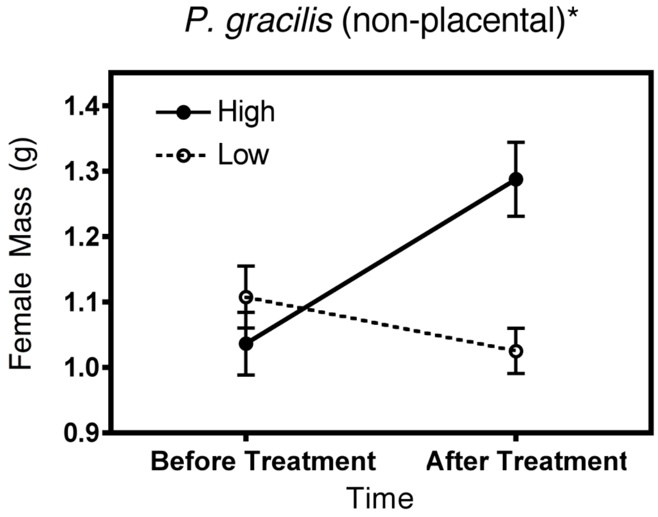


Figure 2.1. Schematic of experimental design. For each species, 15 days of data were collected before the experimental treatments began. Treatments began when approximately half of each species was switched to low food, and lasted 30 days, or approximately one gestation period. Thus, offspring born within the treatment period were initiated before the treatment began, allowing us to see the effect of food level on already developing offspring. Analyses divide data into "pre," "post1," and "post2" time periods.



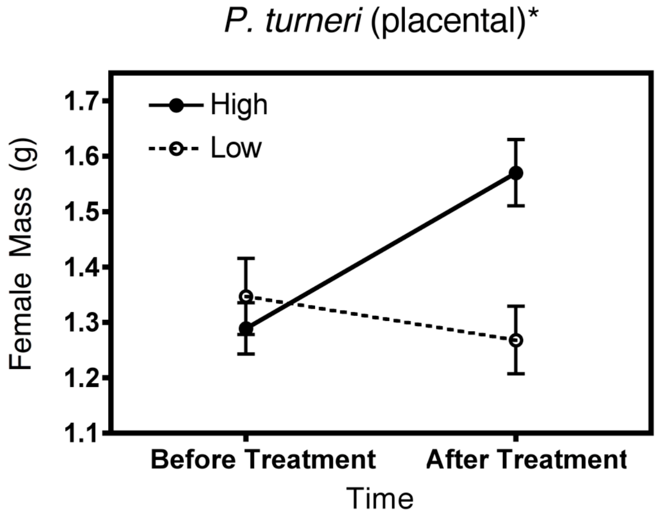


Figure 2.2. Female size immediately before and after the 30 day food treatments. Low food females of both speciesweighed significantly less than their high food counterparts at the end of the experiment, despite no initial differences in mass. At the end of the experiment, non-placental *P. gracilis* females were 124% of their mass at the start of the food treatments. Low food *P. gracilis* females weighed 92% of their initial mass. In placental *P. turneri*, high food females were 122% and low food females were 94% of their initial mass.

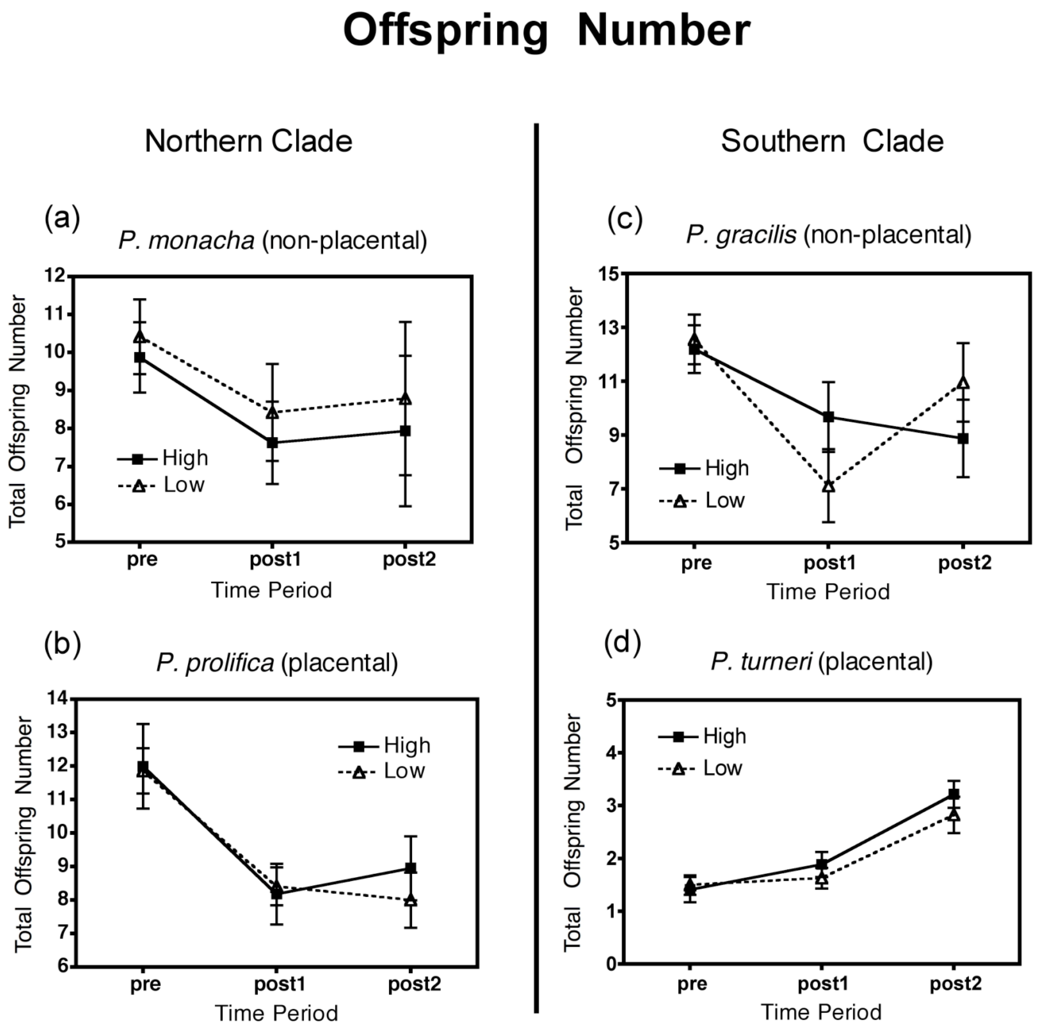


Figure 2.3. Offspring number over time in high and low food in the northern (a & b) and southern (c & d) clades. Times marked “pre” represent the 15 days prior to treatment start. “post1” represents days 1-15 of treatment, and “post2” represents days 16-30 of treatment. No significant differences were found between food groups in any species.

Offspring Mass

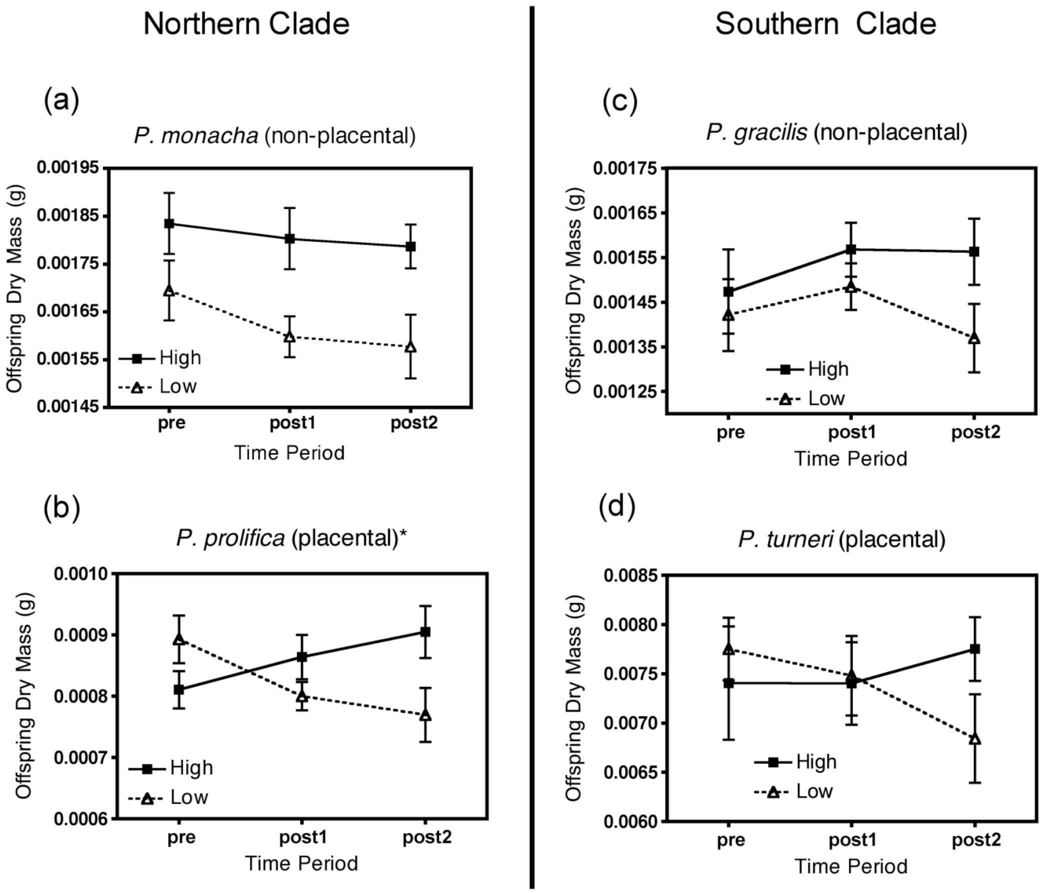


Figure 2.4. Offspring mass over time in high and low food in the northern (a & b) and southern (c & d) clades. In the previous study on the northern clade, high and low food females from placental *P. prolifica* differed significantly in offspring dry mass over time; offspring from low food females show a decrease in mass, while those from high food females show an increase (b). In the present study, placental *P. turneri* shows a similar, but non-significant trend (d).

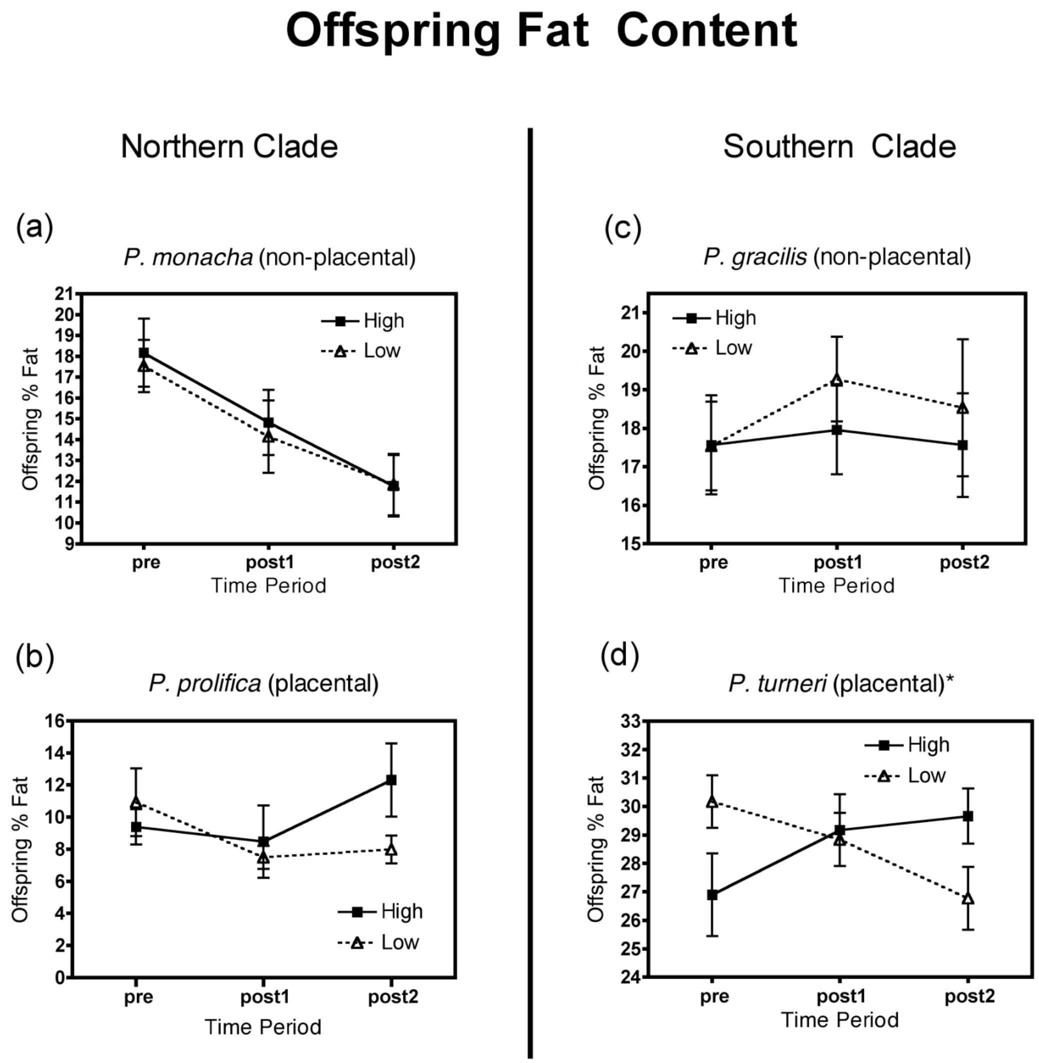


Figure 2.5. Offspring lipid content over time in high and low food in the northern (a & b) and southern (c & d) clades. High and low food females from placental *P. turneri* differed significantly in offspring lipids over time; offspring from low food females show a decrease in lipid content, while those from high food females show an increase (d). In the previous study a similar, but non-significant trend was found in placental *P. prolifica* (b).

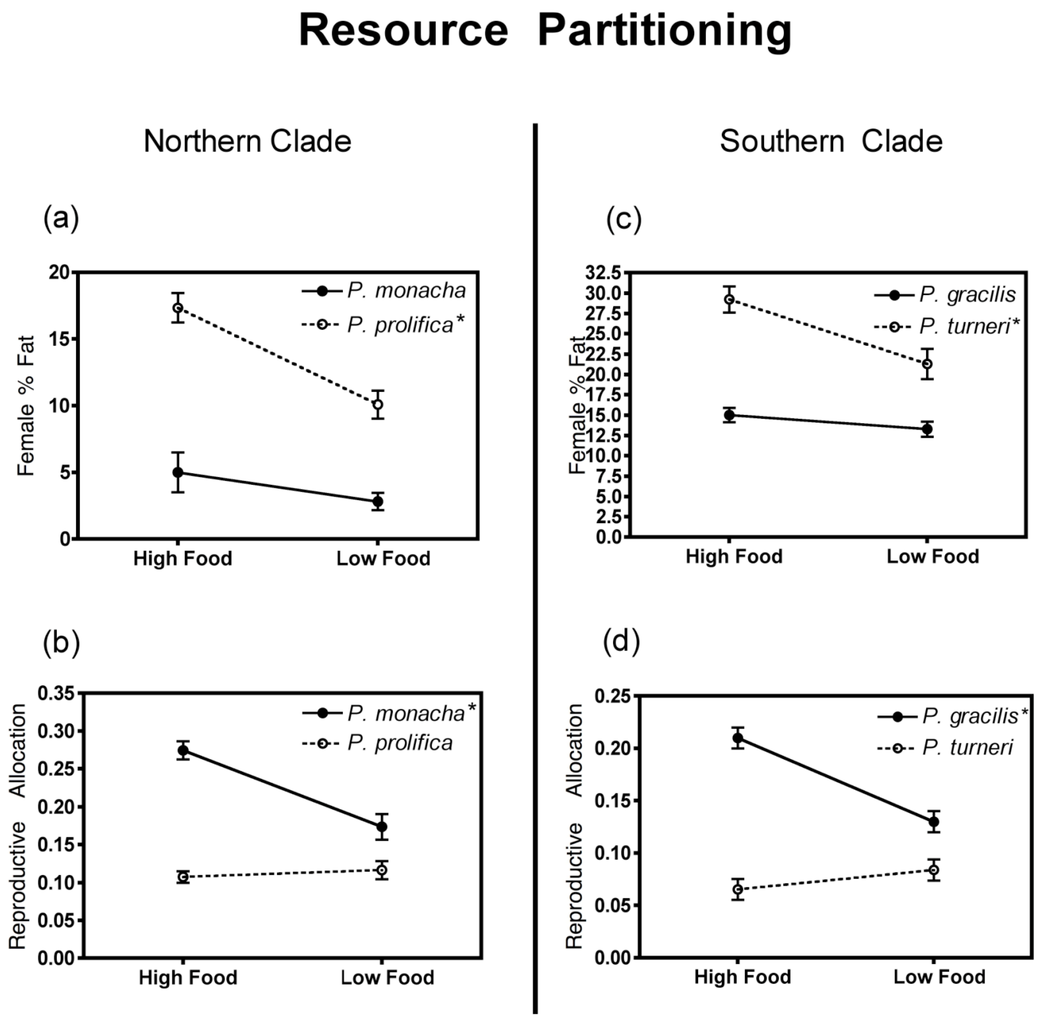


Figure 2.6. Female lipids and reproductive allocation in the northern (a & b) and southern (c & d) clades. Both clades show the same, significant trends. Placental females draw down fat reserves (a & c) in order to maintain reproductive allocation (b & d). Non-placental females maintain fat reserves (a & c) at the expense of reproductive allocation (b & d).

CHAPTER 3:

LINKING REPRODUCTION, LOCOMOTION, AND HABITAT USE IN THE TRINIDADIAN GUPPY, (*POECILIA RETICULATA)*

SUMMARY: Lab studies have shown that pregnancy reduces locomotor ability, increases predation risk, and may translate into a reduction in fitness. Understanding costs of pregnancy on locomotion is thus important in understanding the evolution of reproductive mode and associated life history traits. We investigate the effect of reproductive allocation on locomotor performance, and how this effect translates to habitat use in Trinidadian Guppy (*Poecilia reticulata*) populations that exist in either high or low predation regimes. Female guppies were collected from two high and two low predation streams. Presence of predators and small-scale variation in water flow velocity in the capture localities were recorded. We predicted that females with higher reproductive allocations would be less streamlined, have an inhibited escape response and a higher tailbeat frequency and amplitude when swimming a fixed speed, and would change habitat use to offset these costs. To verify that females collected in different sections of the drainages do exhibit differences in locomotor performance, fish were transported to a nearby lab where escape response and tailbeat frequency and amplitude while swimming a fixed speed were filmed. Immediately following performance trials, females were sacrificed and preserved for dissection to garner reproductive data. We found that high levels of reproductive allocations are associated with a less streamlined shape. Reproductive allocation is negatively correlated with escape velocity and an increase in tailbeat amplitude*frequency when swimming a fixed speed. Reproductive Allocation was not a predictor of habitat selection based on proximity to predators, but females with higher RAs did use habitat with lower water flow velocity.

INTRODUCTION

A central tenet of life history theory is that traits contributing to fitness are linked to one another through trade-offs. The balance of these trade-offs can have a large impact on lifetime reproductive success. An example of this can be seen in the trade-off between current and future reproduction. Investment into reproduction in the present may detrimentally affect future reproduction via factors such as reduced female condition or shortened lifespan (Williams 1966). If the cost associated with a large investment into current reproduction outweighs the benefit in terms of fecundity, then the balance will tilt toward lower investment into reproduction earlier in life. However, external factors such as predation may tilt the balance in the other direction. If a female has a low probability of surviving to the next reproductive season, then a large investment into reproduction earlier in life will be favored. The relationship between these traits, in the context of the environment the organism lives in, will define how their evolution plays out in nature. Understanding the costs and benefits of the variations we see in reproductive strategies will give insight into their evolution and maintenance.

One example of this can be seen when studying the transition from egg-laying to livebearing. A suggested benefit of livebearing is that the female has more control over temperature regulation during development because the mother can more easily behaviorally regulate their temperature (Shine 1983). Additionally, if the eggs are particularly susceptible to predation while in the nest, carrying the eggs internally may reduce this predation pressure (Shine & Bull 1979). Of course, carrying eggs for the entire course of embryo development comes with potential costs. While the female may no longer be bound to her nest, retaining the eggs may delay the initiation of another brood and lead to a decline in fecundity (Tinkle 1969). The physical burden of pregnancy may also impede mobility, creating another cost (Ghalambor et al. 2004, Shine 1980, Shine & Bull 1979, Walker et al. 2005).

This latter cost has been well documented in a diversity of livebearers. In the viviparous lizard *Lacerta vivipara*, gravidity causes a significant reduction in sprint speed (Bauwens & Thoen 1981). Shine (1980) found that running speeds in six species of Australian scincid lizards were reduced 20-30% when gravid. Gravid garter snakes show a decrease in speed, distance crawled, and total time crawled during laboratory performance trials (Seigel et al. 1987). In humans, pregnant women show decreased pelvic and thoracic rotation when walking, resulting in a significant reduction in comfortable walking velocity (Wu et al. 2004). It is not simply the presence or absence of pregnancy that affects locomotion; the mass of the reproductive tissue also plays a role. As gestation progresses, embryos increase in size and mass due to an increase in water weight and in some species, additional allocation of resources by the mother (Banet unpublished data, Ghalambor et al. 2004). Critical swimming speed, a measure of endurance, decreases as pregnancy progresses (and presumably the weight and size of the embryos increases) in mosquito fish (*Gambusia affinis*) (Plaut 2002). Ghalambor et al. (2004) found that stage of pregnancy was negatively correlated with maximum velocity, distance traveled, and acceleration in guppies (*Poecilia reticulata*). A subsequent study showed that a decrease in the aforementioned variables increases a guppy's predation risk when exposed to a natural predator (Walker et al. 2005).

Behavioral changes may reduce the locomotor costs incurred during pregnancy, either by reducing energy expenditure or modifying anti-predator tactics. For example, pregnant females may rely on crypsis rather than fleeing when a predator approaches, as seen in *Lacerta vivipara*. Gravid females of this species allow human predators to approach more closely before fleeing, indicating an increase in cryptic behavior (Bauwens & Thoen 1981). Another strategy a pregnant female may use to reduce locomotor costs of pregnancy is to avoid areas where predation risk is highest. Bighorn sheep (*Ovis canadensis*) live in mountainous habitat with precipitous slopes, allowing them to detect and escape predators. Optimal feeding areas are often at the base of these slopes, where predation risk is higher. Berger (1991) found that bighorn sheep ewes that did not have young with them toward the end of a reproductive season (who were presumably in late stages of pregnancy) spent less time in feeding areas where they were at greater risk of predation, suggesting that predation pressure may override nutritional demand. An examination of lizard studies shows that species with a lower relative clutch mass (RCM, proportion of body mass devoted to reproduction) are more often active foragers, while those that have higher RCMs are more often sit and wait predators, which may help reduce net energy expenditure when feeding (Huey & Pianka 1981, Magnusson et al. 1985, Vitt & Congdon 1978, Vitt & Price 1982), though Roff (1992) points out that because there is a high correlation between foraging mode and family, phylogenetic relationships may be confounding these data.

Few studies have directly examined the relationship between reproduction, locomotion, and behavior. Brodie (1989) examined this link in Northwest garter snakes

(*Thamnophis ordinoides)*. Females with higher RCMs had reduced sprint speeds and crawled shorter distances along a racetrack when prodded. These same females became less confrontational when threatened and showed an increased tendency toward crypsis in behavioral trials conducted in a laboratory setting. Husak (2006) found that female collared lizards (*Crotaphtus collaris*) had slower maximal sprint speeds when gravid, and were slower in nature when foraging and escaping predators. Gravid females in this study used habitat closer to refugia, but did not become more cryptic. The study scored gravidity as dichotomous trait, and did not consider variation in relative clutch mass.

Here we expand on previous studies by considering how variation in the proportion of body weight devoted to reproduction affects locomotion and behavior (habitat use) in the field, and how this behavior varies across predation regimes. To do this we examined populations of guppies (*Poecilia reticulata*), a livebearing fish that can be found in habitats varying in predation pressure. We predicted that (1) females with higher reproductive allocations (RA, the fish literature equivalent to RCM) will have a less streamlined body shape, (2) females with higher reproductive allocations will have reduced locomotor ability in both burst and prolonged swimming, (3) In high predation locations, females with higher reproductive allocations will preferentially use regions of available habitat away from predators, leading to a greater behavioral difference in habitat use between females with high vs. low reproductive allocations than is seen in low predation locations and (4) females with higher reproductive allocations will preferentially use regions of the available habitat with the lowest water flow velocity.

METHODS

Guppies (*Poecilia reticulata)* are small, livebearing fish. In Trinidad, they can be found in freshwater stream drainages that have discrete predation regimes separated by a barrier waterfall. Downstream from the base of the waterfall, guppies coexist with a number of larger fish species (e.g., *Crenicicichla alta, Hoplias malabaricus, Astayanax dentatum*) that are capable of preying on adult size classes. The waterfall excludes these predators from reaching areas upstream. Guppies in these upstream localities live in a low predation environment. Previous work has shown that high and low predation guppies have evolved different life histories. High predation fish show the suite of life history traits expected to evolve in high extrinsic mortality; they are smaller and younger at maturation, produce more, smaller offspring, and important for this study, they have a larger reproductive allocation than low predation fish (Reznick & Endler 1982).

This study focused on two streams, the Aripo and El Cedro (a tributary of the Guanapo). In the Aripo drainage, guppies naturally occur in both the high and low predation habitats. The Naranjo tributary was used as the low predation habitat in the Aripo drainage. The Naranjo is a narrow and structurally complex stream, creating refuges from water flow velocity. The high predation Aripo habitat used in this study was wider and included large open areas where schools of predators could be seen. For simplicity these will be referred to as Aripo low (AL) and Aripo high (AH). El Cedro high (CH) and low (CL) predation habitat were both similar to the to the AL habitat; the stream was narrow with rocky outcroppings that provided structure and refuge to the guppies (widths, based on three haphazard measurements at each site: AH: 699 +/- 44.09

cm, AL: 195.33 +/- 21.14 cm, CH: 225 +/- 43.59 cm, CL: 176 +/- 49.15 cm). Visual examination of El Cedro high predation habitat reveals fewer predators than is seen in the Aripo high predation locality.

Specimen Collection

Guppies were collected from three sites within each of the four locations described above, making a total of 12 study sites. We chose sites that exhibited variation in water flow velocity and were relatively contained by riffles on each side. Each site was divided into sections. Females within each section were captured with hand nets, and water flow velocity was measured at three haphazardly picked spots within each section. Originally, sections were to be made by dividing the habitat into square sections of even size, as done in a previous study examining habitat use in salmonids (Morinville & Rasmussen 2006). In practice however, the microvariation in water flow velocity caused by the structurally complex nature of our study sites resulted in many grid sections containing water that was flowing at vastly different velocities, introducing error into our measures of the water flow velocity each female was using. Another problem with this method was that unlike the previous studies, which used electrofishing during sample collection, we collected fish with handnets. When using handnets more care must be taken to ensure that the fish are not perturbed to such an extent that they leave the grid section. These problems prompted us to develop an alternative method of dividing the habitat into sections. When observing guppies using the habitat naturally, they tended to gather in loosely aggregated shoals, each of which stayed in a relatively contained section of the habitat. We divided the habitat into sections based on how these shoals were using it and measured the area of each section after collection was completed. This solved both of the aforementioned problems; when divided this way the water flow velocity within each section was relatively uniform and fish rarely left the section, even when perturbed with hand nets. Before sample collection in high predation locations, we recorded the presence/absence of predators in each of the sections by observing the site for 20 minutes. If a predator entered a section during the observation period, it got a positive rating.

After collection, females were transported to a nearby lab and allowed to acclimate for one day. Escape response trials were conducted on day two; prolonged swimming trials were conducted on day three. Immediately after locomotor performance trials were completed (no more than 72 hours after collection from the field), the female was sacrificed and dissected in order to get reproductive information.

Escape Response Trials

All teleost fish exhibit a stereotyped fast-start response when startled. The fish curves its body into a "C" shape and rapidly accelerates away from the stimulus (Weihs 1973). Escape response trials were conducted following methods similar to those of Ghalambor (2003). Individual females were moved to an escape arena and left to acclimate for 10 minutes. The arena consisted of a circular mesh enclosure with a 16 cm diameter, which was situated in a 40.94x31.75 cm^2 tank with a 5 cm grid marked on the bottom of it. Two halogen lights were situated around the arena to provide adequate illumination. To minimize vertical movement during the escape the tank was filled with water to a depth of only three cm. To initiate the escape response a 21.8 g weight was dropped through a 110 cm long PVC pipe, which was suspended several millimeters above the water surface, but outside the mesh enclosure that housed the fish. The PVC pipe prevented the guppies from seeing the stimulus before it broke the water, reducing the likelihood that that the fish were responding to stimuli other than the weight breaking the water surface. The stimulus was dropped when the female was near the center of the mesh enclosure to avoid wall effects, and was not moving to ensure she had no momentum going into the escape. A maximum of three trials was attempted for each female. If there was no response to the stimulus after the third attempt, we did not collect escape response data for that individual. Response rates are reported in the results section. If multiple trials were conducted, we waited 10 minutes between each release of the stimulus weight. Because water temperature can affect swimming performance, all trials were conducted at 22ºC +/- one degree, which is the temperature of the water the fish were housed in during the acclimation period. Trials were filmed from above at 200 frames per second with a Prosilica GE680 VGA CCD camera.

Prolonged Swimming Trials

To get an indicator of how the physical burden of pregnancy affected a female during prolonged swimming, tailbeat amplitude and frequency was measured at three different swimming velocities. A subject was transferred to a water flow tunnel with working dimensions of approximately 45x45x110 mm^3 and allowed to acclimate for 10 minutes. The chamber that contained the fish was connected to a pipe that formed a closed circuit. A propeller forced water through the circuit and had a dial that could be adjusted to produce a range of water flow velocities. The tunnel was calibrated by filming a neutrally buoyant particle moving through the swim chamber three times each at five different dial settings. A regression line was fitted to this data, which allowed us to relate different settings on the dial to different water flow velocities. To create a more laminar flow within the chamber, a small 'honeycomb' was constructed out of standard size drinking straws and placed on either side of the swimming chamber. Water flow velocity was slowly increased to trial speeds of 10, 12.5, and 15 cm/second. These speeds are within the range of speeds that guppies were found using in the wild in this study. Trials were filmed at 200 frames per second. A mirror placed under the flow tunnel at an angle of 45° allowed us to film the ventral perspective of the fish. To minimize error due to microvariation within the water tunnel, data were only taken when the fish was in the central section of the length and the lower half of the height of the tunnel. Data collection for an individual at a given speed was considered complete when we recorded 5 full tailbeats of swimming where the fish did not move more than 5% of its body length forward or backwards. Trials were conducted at 22ºC +/- one degree.

Reproductive Allocation

We used wet reproductive allocation (RA) as a measure of the physical reproductive burden associated with pregnancy. It is important to note that our measure of RA was made using wet weights. Guppy embryos lose dry weight during gestation, but their wet weights increase nearly 4-fold over the course of development (Ghalambor et al. 2004). Because of this, dry weight is not an accurate indicator of the physical burden a female experiences when alive. After the performance trials, females were sacrificed using an overdose of MS-222 (Sigma Aldridge) and preserved in a solution of 5% formalin. Preservation of samples in 5% formalin does not significantly affect the wet weight of the samples (David Reznick, unpublished data). Samples were then transported to the University of California, Riverside. There we measured standard length and width at the widest point to the nearest hundredth of a millimeter of each female using digital calipers, in order to calculate the fineness ratio (the ratio of standard length to width at the widest point) (Scarnecchia 2006). Females were then dissected. Fineness ratio is a measure of streamlining commonly used in the field of aeronautics. A ratio of approximately 4.5 produces the least drag (von Mises 1949). Fish biologists have employed fineness ratio to examine the variation in drag experienced by fish of differing morphologies. High speed, long distance swimmers such as Tuna exhibit fineness ratios near 4.5 which reduces their energetic expenditure, while species that do little sustained swimming such as many reef fish tend to have higher fineness ratios (Webb 1975). Embryos and all associated reproductive tissues were separated from the remainder of the female somatic tissue. Wet weights of each was measured and recorded to the nearest hundred-thousandth of a gram with a Mettler AE163 analytical balance.

Analysis

Videos were analyzed using ImageJ 1.38x software. Walker (2005) showed that escape performance in the first 22 milliseconds of a fast start was positively related to the probability of predator evasion. Thus, we digitized the movement of the fish's center of mass during escape response trials up to 20 milliseconds (4 frames) after the escape began (the closest approximation possible based on our frame rate of 200 frames per second, which gave a resolution of 5 milliseconds). Center of mass in guppies is estimated to be located 35% of the total length of the fish (Ghalambor et al. 2004). Distance moved in the first 20 ms of the escape was used as a proxy for velocity. In the prolonged swimming trials, tailbeat amplitude was recorded as the average amplitude over 5 full tailbeats. The time it took for a fish to complete 5 full tailbeats was converted into tailbeats/second before it was analyzed. From this, we calculated tailbeat amplitude*frequency, which is used as an indicator of kinematic change in fish swimming studies (Webb 1993). A higher tailbeat amplitude*frequency indicates that a fish is expending more energy while swimming.

We first describe the general biology of our sample. A stepwise multiple regression was used to analyze how wet RA changed over the course of development, including predation, drainage, and embryo number as possible factors in order to control for their effects. Because we had an a priori prediction that high predation fish would have larger reproductive allocations, a one-tailed t-test was used to detect differences in wet RA between predation regimes. We analyzed the effect of wet RA on fineness ratio, escape velocity, tailbeat amplitude*frequency, proximity to predators, and habitat water flow velocity. We also examined the relationship between predation regime and habitat water flow velocity. A stepwise multiple regression was used to examine the effect of RA on fineness ratio, escape velocity, and habitat flow velocity, including drainage, predation regime, and total length as possible factors in order to control for their effects. A linear mixed regression model was used to examine the relationship between wet RA and tailbeat amplitude*frequency at the three speeds. We used a two tailed t-test to examine the relationship between wet RA and a female's proximity to predators, and to look at how predation affected the habitat water flow velocity used. Number of subjects varies between analyses because not all females performed during swimming trials (i.e. they were non-responsive in the escape trials, or refused to swim.

If females with higher reproductive allocations have a less streamlined body shape, the females with the lowest RAs will have fineness ratios close to 4.5, and the fineness ratio will move away from a value of 4.5 as wet RA increases. If increased reproductive allocation has a detrimental effect on locomotor ability, then I predict females with higher wet RAs will have a lower velocity during escape response and a higher tailbeat amplitude*frequency when swimming at a fixed speed. If the burden of pregnancy reduces escape velocity, then I predict females with higher RAs will be found in lower water velocities a disproportionate amount of time. Finally, if the increased burden of pregnancy constrains prolonged swimming, then I predict that females with higher wet RA will frequent habitats with lower water flow velocity.

RESULTS

General Biology: Wet reproductive allocation increased as stage of development progressed, indicating a larger physical burden exists at later stages of pregnancy (multiple linear regression with embryo number and predation regime included as an independent factors to control for differences: $t_{183} = 6.922$, $p < 0.001$. Drainage was not needed in the analysis). As previous studies have shown, high predation fish in this study had significantly higher wet RAs (Fig. 3.1: one-tailed t-test: $t_{188} = 3.583$, $p < 0.001$. Drainage was not needed as a covariate).

Fineness Ratio: Fineness ratio, a measure of streamlining, is the ratio of standard length to width at the widest point (Scarnecchia 2006). Fineness ratio was negatively correlated with wet reproductive allocation (Fig. 3.2a: linear regression: $t_{188} = 54.850$, $p<0.001$). A fineness ratio of approximately 4.5 has the lowest pressure drag. The regression equation predicts that a female will be the most streamlined with a reproductive allocation of approximately 13.24%. The range of RAs found in this study was from 5.3 to 37.92%, meaning the RA with the most streamlined shape falls in lowest quartile of values. The mean RA found in this study was 18.07% (standard error = 0.43), indicating the average pregnant female has a RA value higher than that which is predicted to be most streamlined.

Escape Response: Fish from the Aripo drainage responded to the stimulus 100% of the time, which was significantly higher than the 91.67% response rate seen in El Cedro drainage (t_{196} = 2.649, p = 0.009). There was no significant difference between responsiveness in high and low predation El Cedro females (t_{118} = -1.725, p = 0.087), though low predation fish did show a trend for higher responsiveness (95.52% vs. 86.79% in high predation). Distance moved within the first 20 milliseconds of an escape response was used as a proxy for velocity. As mentioned above, this timeframe has been shown to be ecologically relevant in previous studies (Walker et al. 2005). Females with higher reproductive allocations had significantly slower escape responses (Fig. 3.3: multiple linear regression with drainage included as an independent factor to control for differences: t_{172} = -2.466, p = 0.015. Predation regime was not needed in the analysis).

*Tailbeat Amplitude*Frequency:* The product of tailbeat amplitude and frequency is used as an indicator of kinematic change in fish swimming studies (Webb 1993). As described above, tailbeat amplitude*frequency was measured at three different speeds. Because data taken at each of these three speeds are not independent from one another, we used a linear mixed model regression to combine these into a single analysis. Linear mixed models do not assume data are independent, and can take intraclass correlations into account. Female guppies with higher RAs had significantly higher tailbeat amplitude*frequency measurements (Fig. 3.4: linear mixed model regression: $F_{1,154}$=9.618 $p<0.001$. Drainage and predation regime were not needed in the analysis), indicating that they had to work harder than females with lower RAs to maintain a fixed speed.

Proximity to Predators: No female occupied sections of the stream that predators were recorded using. We instead based our analysis on whether predators could be found using contiguous sections of the stream. In the AH population, there was no difference in RA between sections of the stream that had predators in contiguous sections, and those that did not (Fig. 3.5: two-tailed t-test: t_{30}=1.661, p=0.107). In the CH population, no female used sections of the streams with predators in contiguous sections, so no analysis was performed. This is potentially an artifact of the CH locality having fewer predators overall.

Habitat Water Flow Velocity: When all populations were pooled, our analysis showed that females with higher reproductive allocations used lower water flow velocities (Fig. 6a: multiple regression with drainage and predation regime included as factors: t_{185}=-2.108, p=0.036). However, this analysis showed drainage, predation, and the interaction between the two were all significantly correlated with habitat water flow velocity. Because of this, we performed additional analyses. In the low predation localities there was not a significant drainage effect, thus these were pooled for analyses. This result mirrors the result when pooled; females with higher reproductive allocations were using lower water flow velocities (Fig. 6b: multiple regression: t_{105}=-2.433, p=0.017). High predation localities showed a significant effect of drainage, so they were separated for analysis. Neither population showed a significant effect of RA on habitat flow velocity (Fig. 6c and 6d: linear regressions: AH: t_{30} = 1.323, p = 0.196; CH: t_{49} = 0.544, p = 0.589). We instead found that all high predation fish of both localities restricted their activities to regions of low water flow velocity. The mean velocity used in these habitats was significantly slower than in the habitat used by their low food counterparts (Fig. 7a: t-tests, equal variances not assumed: Aripo: $t_{48.996} = -5.159$, $p < 0.001$; El Cedro: $t_{106.879} = -2.424$, $p = 0.017$), despite no difference in mean water flow velocities between our high and low predation sites within each drainage (Fig. 7b: t-test performed on mean water flow velocity of all sections within a site, weighted by section area: Aripo: $t_{25} = -0.487$, $p = 0.630$; El Cedro: $t_{38} = 1.295$, $p = 0.203$).

DISCUSSION

Examining reproduction, locomotor performance, and habitat use within individuals over a short time scale allows us to see how a cost of reproduction manifests behaviorally in a natural setting. We had four predictions going into this study. First, we predicted that females with higher wet reproductive allocations would have less streamlined body shapes. Second, we predicted that females with higher reproductive allocations would have reduced locomotor performance in both burst and prolonged swimming. Third, we predicted that females in high predation localities would preferentially use regions of habitat away from predators, leading to a greater behavioral difference between females with high vs. low levels of reproductive allocation. Finally, we predicted that females with higher reproductive allocations would preferentially use regions of the available habitat with the lowest water flow velocity.

We found moderate support for our first hypothesis. Our regression line predicts that a RA of approximately 13.24% will yield the most streamlined shape. While some pregnant females had smaller values than this, it still falls into the lowest quartile of the values we measured, and is below the mean RA found in our sample. This means that for most females, pregnancy confers an overall decrease in streamlining. Because wet RA increases over the course of gestation, it is likely that a female will attain the most streamlined shape early in gestation, and will move farther away from the ideal fineness ratio as the pregnancy progresses. However, the relationship between drag and fineness ratio is not linear (Fig. 3.2b). As fineness ratios move below 4.5, drag increases only minimally until the ratio falls below approximately three. Animals with fineness values lower than this experience a steep increase in drag. Above 4.5, drag increases gradually, such that fineness ratios six or seven yield only minor changes in drag (von Mises 1949). The range of fineness ratios seen in this study was from 3.32 to 5.37, meaning that the change in pressure drag across different reproductive allocations was quite small. Deeper bodied species (with a lower fineness ratio when not pregnant) would therefore be more likely to suffer a larger locomotor cost of pregnancy due to increased pressure drag, because smaller decreases in fineness ratio would cause a larger increase in pressure drag. Despite this, changes in locomotor performance seen in this study are most likely a result of the minor increase in drag associated with higher wet RAs; most aquatic organisms are neutrally or only slightly negatively buoyant, meaning that the increased mass alone would have little effect on locomotor performance. Drag plays a particularly important role in aquatic locomotion, because water has a much higher viscosity than air (Biewener 2003).

Our second hypothesis was also supported. An increase in wet RA negatively affected both burst and prolonged swimming performance. As wet RA increased, escape velocity within the first 20 milliseconds of a response decreased significantly. This corroborates an earlier study by Ghalambor et al. (2004), which looked at the effect of reproductive allocation on the escape responses of lab-reared F2 guppies. As described previously, a reduction in escape performance within this time frame decreases the probability of survival when attacked by a natural predator (Walker et al. 2005). However, direct comparison to Walker et al.'s study cannot be made because their measure of escape performance was a composite variable that included maximum acceleration. We were unable to get good measures of acceleration due to equipment constraints. Because acceleration is a double derivative, it has a higher level of noise than other measures, and is highly dependent on the system used (magnification, frame rate, smoothing technique) (Harper & Blake 1989). Walker et al. filmed their trials at 1000 hertz, while our camera had a maximum frame rate of 200 hertz. Additionally, their study used a quintic spline to smooth the acceleration data. Our frame rate only allowed us to collect four data points for each individual over the relevant time period, so this smoothing technique could not be used. A conversion of their variable d_{net} (a measure of distance traveled over time) into mean velocity allows a rough comparison between the two studies. Fish in the previous study (Walker et al. 2005) had a mean velocity of 23 +/- 1 cm/s. The mean velocity of those that escaped a predator strike was 24.4 +/- 1.5 cm/s, while those that were unsuccessful had a mean velocity of 20.7 +/- 2.2 cm/s. Fish from the present study exhibit higher means; all populations combined have a mean velocity of

32.88 +/- 0.91 cm/s (individual populations: AH: 32.49 +/- 2.14 cm/s, AL: 29.01 +/- 1.52 cm/s, CH: 35.07 +/- 1.94 cm/s, CL: 34.40 +/- 1.64 cm/s). This difference could be due to a ‘training’ effect from being in the field; Walker et al.’s study used lab-reared and aquarium trade guppies, which were presumably housed in tanks with relatively still waters and no predators. Differences between this type of environment and a natural environment have the potential to trigger plastic responses in muscle composition and performance, which could contribute to the differences observed in mean velocities. If this training effect does indeed exist, it makes inferences about the ecological relevance of our measures of escape velocity difficult. To properly address this, we would need to know if the performance of the predators in our field sites differed from those in the Walker et al. study, as we see in our guppy performance data.

The second component of hypothesis two was supported as well; prolonged swimming was negatively affected by an increase in wet reproductive allocation. As wet RA increased, there was a significant increase in tailbeat amplitude*frequency when swimming a fixed speed, indicating that a female with a higher RA would have to expend more energy to maintain the same speed as a female with a lower RA. This contrasts with the Plaut (2002) study on *Gambusia affinis*. Plaut measured critical swimming speed (a measure of endurance) and tailbeat amplitude and frequency. He found that as pregnancy progressed, there was a significant decrease in critical swimming speed; however, he did not see any difference in tailbeat amplitude or frequency. He thus concluded that the cost of pregnancy on locomotion was most likely physiological, where the embryos deplete the oxygen supply of the female, leaving less oxygen to fuel locomotion. It is possible Plaut's findings differ from the present study because his sample size was much smaller (n=8, compared to n=157).

Hypothesis three was not supported. In the Aripo drainage, wet reproductive allocation did not significantly influence a pregnant female's proximity to predators, nor were there any compelling trends. In El Cedro drainage, analyses could not be performed because no female used habitat sections that were the same as or contiguous to sections the predator was seen using. This is likely because the CH locality had fewer predators overall (Banet, personal observation), reducing the probability that predators and guppies will be using the same habitat.

Our final hypothesis, that females with higher reproductive allocations would preferentially use regions of the available habitat with the lowest water flow velocity, was supported in low predation habitats. Females with higher reproductive allocations used areas with significantly slower water flow velocities. No relationship was found between reproductive allocation and water flow velocity in high predation localities. However, in both drainages, high predation females used significantly slower water flow velocities than did females from low predation habitats despite the availability of habitat with higher water velocities. We suspect females in high predation habitats restrict their activities to slow moving margins and relatively enclosed pools that may not be suitable for larger fish, thus obscuring the effect of RA on habitat water flow velocity. This restriction of habitat use may represent a different facet of our prediction. If predators were removed from the system, females may expand the range of water flow velocities used revealing an effect similar to that seen in low predation.

To fully understand the implications of the changes we see in habitat use, it would be necessary to identify related trade-offs. Anecdotally, faster moving water is the preferred feeding ground for female guppies (David Reznick, personal communication). All of the guppies in this study collected in water flow velocities faster than 15 cm/s were observed actively feeding on small particles in the water column as they flowed by. A study that analyzed gut contents of females collected at different water flow velocities, as well as quantification and description of the food available at different velocities would shed light on the validity of this trade-off.

Perhaps one of the most interesting aspects of this work is its application to the evolution of reproductive modes within livebearers. Timing of provisioning in livebearers follows a continuum. At one end of the continuum are lecithotrophic species, which are essentially like egg-layers that retain their eggs for the course of gestation. The eggs are fully yolked before fertilization, and after fertilization little or no nutrients are allocated to the developing embryo. At the other end of the continuum are matrotrophic species. Matrotrophs start with a small egg at fertilization, and continue to allocate nutrients throughout gestation. Matrotrophs in the family Poeciliidae (of which guppies are a lecithotrophic member) do this by means of a placenta. Matrotrophy has evolved from lecithotrophy three independent times in the genus *Poeciliopsis* (Reznick et al. 2002), as well as having multiple independent origins in other taxa (Blackburn 1992). Despite these multiple origins, there is no confirmed explanation for its evolution. In both reproductive modes, the wet weight of embryos increases over the course of development. However, the magnitude of change in a matrotroph can be much larger due to the small initial egg size (Banet, unpublished data). A consequence of this is that matrotrophs will have a lower mean reproductive allocation over the course of gestation, assuming that female size and terminal offspring size/number are equal between reproductive modes. Placental matrotrophy is also often found in concert with a trait called superfetation, where the female can carry broods in multiple stages of development. Superfetation further serves to reduce reproductive allocation because a female never has all of her developing offspring in the largest, most burdensome stage at the same time (Pollux et al. 2009).

At least two studies have looked at correlates between matrotrophy and the suite of other life history traits to generate testable predictions that can be linked back to life history theory. Bassar et al. (unpublished data) examined two independent origins of placental matrotrophy in the livebearing fish genus *Poeciliopsis*. In the northern clade of the genus, placentation is correlated with a decrease in age and size at maturity, and the production of more, smaller offspring. However, in the southern clade the presence of the placenta is instead correlated with an increase in age and size at maturity, and the production of fewer, larger offspring. Of all the life history traits measured, the only consistent correlate with matrotrophy between the two clades was a reduction in reproductive allocation. A similar pattern was found in the fish family Zenarchopteridae (genera *Nomorhamphus* and *Dermogenys*). In *Nomorhamphus*, the presence of the placenta was correlated with the production of more, smaller offspring, whereas in *Dermogenys* the placenta was correlated with having fewer, larger offspring. Again, the only consistent correlate with placental matrotrophy found between the two genera was a reduction in reproductive allocation (Reznick et al. 2007). Further still, Pires et al. conducted a preliminary study (unpublished) and found that placental matrotrophy was significantly correlated with habitats that had faster flowing water. Measures of water flow velocity in this study were based on expert's recollection of the flow velocity at the study sites. Together, this circumstantial evidence suggests that matrotrophy may have evolved as an adaptation to reduce the costs of pregnancy on locomotion without modifying habitat selection.

This study has demonstrated the link between reproduction, locomotor performance, and habitat use. It has also added to the growing list of circumstantial evidence that suggests matrotrophy may have evolved as a way to reduce the locomotor cost of pregnancy by showing that a higher wet reproductive allocation leads to a reduction in locomotor performance, which translates to a potentially costly change in habitat use. Because matrotrophy is consistently correlated with a reduction in reproductive allocation, it may allow a pregnant female to use a wider range of the habitat at a lower cost. Future studies should focus on quantifying the costs associated with modifying habitat use based on reproductive allocation, particularly how resource availability and quality differs between areas with different water flow velocities. Further exploration is also needed to verify the claim that matrotrophy evolved as a way to reduce locomotor costs of pregnancy. The Pires et al. preliminary study mentioned above provides a starting point, and suggests that a larger scale study which quantifies water flow velocity and examines how microhabitat usage affects the water flow velocity and individual is actually experiencing would be a worthwhile next step. Additionally comparative studies that characterize locomotory costs (both kinematic and physiological) of pregnancy on placental and non-placental females are needed.

REFERENCES

Bauwens, D. & Thoen, C. (1981). Escape tactics and vulnerability to predation associated with reproduction in the lizard *Lacerta vivipara*. *Journal of Animal Ecology* **50**, 733-743.

Berger, J. (1991). Pregnancy incentives, predation constraints, and habitat shifts: experimental and field evidence for wild bighorn sheep. *Animal Behaviour* **41**, 61-77.

Biewener, A. *Animal Locomotion* (Oxford University Press, 2003)
Blackburn, D. (1992). Convergent evolution of viviparity, matrotrophy, and specializations for fetal nutrition in reptile and vertebrates. *American Zoologist* **32**, 313-321.

Brodie, E. D. (1989). Behavioral modification as a means of reducing the cost of reproduction. *American Naturalist* **134**, 225-238.

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

Harper, D.G. & Blake, R.W. (1989). A critical analysis of the use of high-speed film to determine maximum accelerations of fish. *Journal of Experimental Biology* **142**, 465-471.

Huey, R. B. & Pianka, E. R. (1981). Ecological consequences of foraging mode. *Ecology* **63**, 991-999.

Husak, J. F. (2006). Do female collared lizards change field us of maximal sprint speed capacity when gravid? *Oecologia* **150**, 339-343.

Magnusson, W. E., de Paiva, L. J., da Rocha, R. M., Franke, C. R., Kasper, L. A. & Lima, A. P. (1985). The correlates of foraging mode in a community of Brazilian lizards. *Herpetologica* **41**, 324-332.

Morinville, G. R. & Rasmussen, J. B. (2006). Does life-history variability in salmonids affect habitat use by juveniles? A comparison among streams open and closed to anadromy. *Journal of Animal Ecology* **75**, 693-704.

Plaut, I. (2002). Does pregnancy affect swimming speed in female mosquitofish, *Gambusia affinis? Functional Ecology* **16**, 290-295.

Pollux, B. J. A., Pires, M. N., Banet, A. I. & Reznick, D. N. (2009). Evolution of placentas in the fish family Poeciliiadae: An empirical study of macroevolution. *Annual Review of Ecology and Systematics* **40**, 271-289.

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

Reznick, D., Meredith, R. & Collette, B. B. (2007). Independent evolution of complex life history adaptations in two families of fishes, live-bearing halfbeaks (Zenarchopteridae, Beloniformes) and Poeciliidae (Cyprinodontiformes). *Evolution* **61**, 2570-2583.

Reznick, D., Mateos, M. & Springer, M. (2002). Independent origins and rapid evolution of the placenta in the fish genus *Poeciliopsis*. *Science* **298**, 1018-1020.

Roff, D. *The Evolution of Life Histories* (Routledge, Chapman, and Hall, New York, 1992)

Scarnecchia, D. (2006). The importance of streamlining in influencing community structure in channelized and unchannelized reaches of a prairie stream. *Regulated Rivers: Research & Management* **2**, 155-166.

Seigel, R. A., Huggins, M. M. & Ford, N. B. (1987). Reduction in locomotor ability as a cost of reproduction in gravid snakes. *Oecologia* **73**, 481-485.

Shine, R. I. (1980). "Costs" of reproduction in reptiles. *Oecologia* **46**, 92-100.

Shine, R. I. (1983). Reptilian Viviparity in cold climates: testing the assumptions of an evolutionary hypothesis. *Oecologia* **57**, 397-405.

Shine, R. & Bull, J. (1979). The evolution of live-bearing in lizards and snakes. *American Naturalist* **113**, 905-923.

Tinkle, D. (1969). The concept of reproductive effort and its relation to the evolution of life histories of lizards. *American Naturalist* **103**, 501-516.

Vitt, L. J. & Congdon, J. D. (1978). Body shape, reproductive effort, and relative clutch mass in lizards: resolution of a paradox. *American Naturalist* **112**, 595-607.

Vitt, L. J. & Price, H. J. (1982). Ecological and evolutionary determinants of relative clutch mass in lizards. *Herpetologica* **38**, 237-255.

Von Mises, R. *Theory of Flight* (Dover Publications, 1949)

Walker, J. A., Ghalambor, C., Griset, O. L., McKenney, D. & Reznick, D. (2005). Do faster starts increase the probability of evading predators? *Functional Ecology* **19**, 808-815.

Webb, P.W. (1975). Hydrodynamics and energetics of fish propulsion. *Bulletin of Fisheries Research Board of Canada* **190**, 1-159.

Webb, P. W. (1993). The effect of solid and porous channel walls on steady swimming of steelhead trout *Oncorhynchus mykiss*. *Journal of Experimental Biology* **178**, 97-108.

Weihs, D. (1973) The mechanism of rapid starting of slender fish. *Biorheology* **10**, 343-350.

Williams, G. C. (1966). Natural selection, the costs of reproduction, and a refinement of Lack's Principle. *American Naturalist* **100**, 687-690.

Wu, W., Meijer, O. G., Lamoth, C., Uegaki, K., van Dieen, J. H., Wuisman, P., de Vries, J. & Beek, P. J. (2004). Gait coordination in pregnancy: transverse pevic and thoracic rotations and their relative phase. *Clinical Biomechanics* **19**, 480-488.

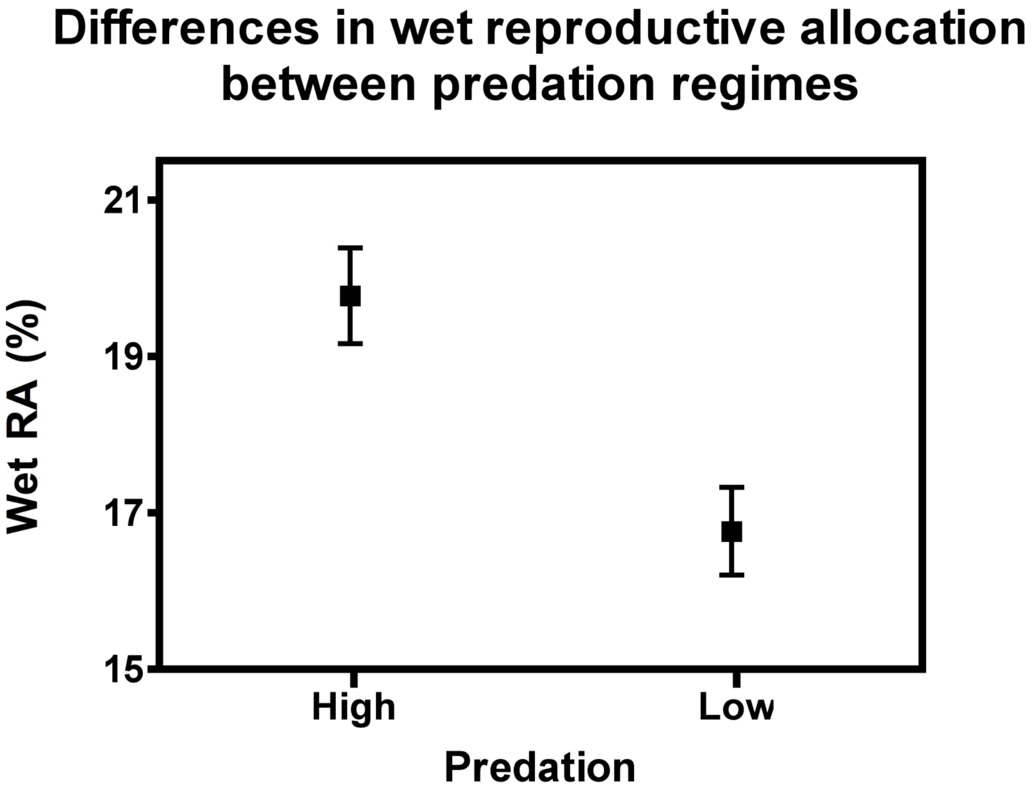


Figure 3.1. Mean wet reproductive allocation (RA) in high and low predation populations used in this study. High predation fish had significantly higher wet RAs than low predation fish.

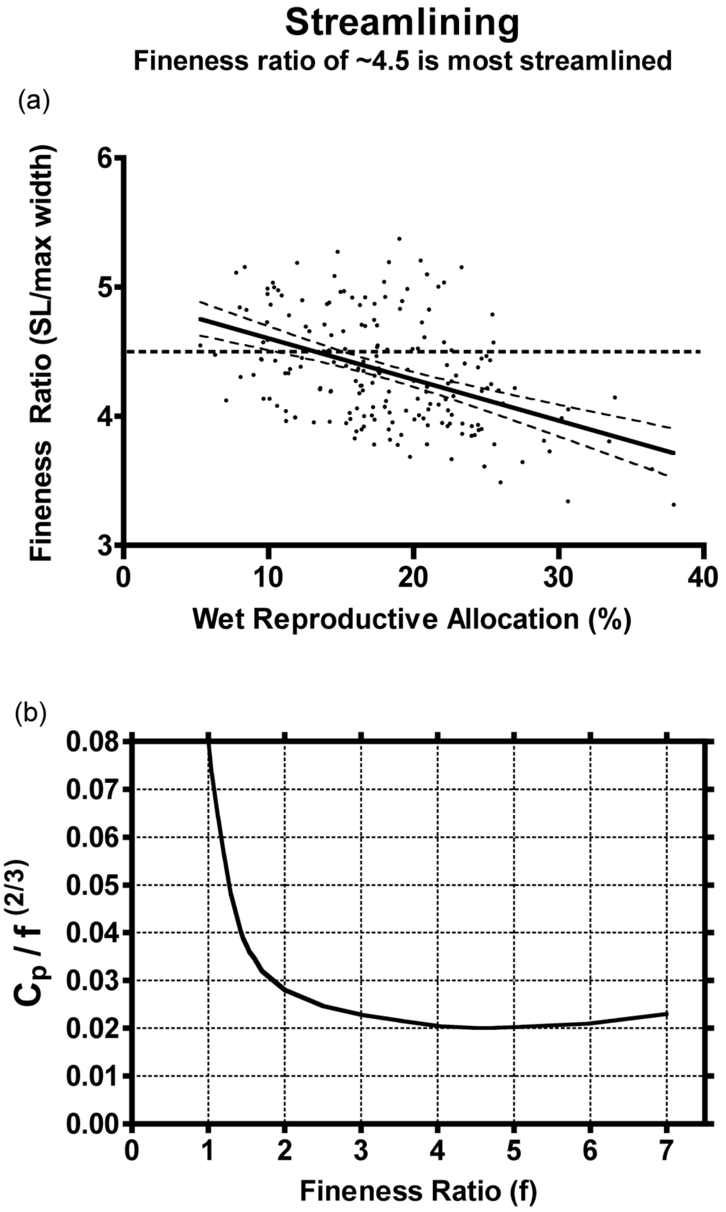


Figure 3.2. (a) Relationship between RA and fineness ratio. The regression equation predicts an RA of 13.24% will be the most streamlined. This value falls in lowest quartile of values and is lower than the mean wet RA. (b) Modified from von Mises 1959. Pressure drag (C_p) divided by the fineness ratio (f) taken to the $^{2}/_{3}$ power is directly proportional to total drag (see von Mises 1959 for detailed explanation). The relationship between fineness ratio and drag is non-linear. In this study, fineness ratio ranged from 3.32 to 5.37, meaning that changes in RA caused only minimal changes in drag.

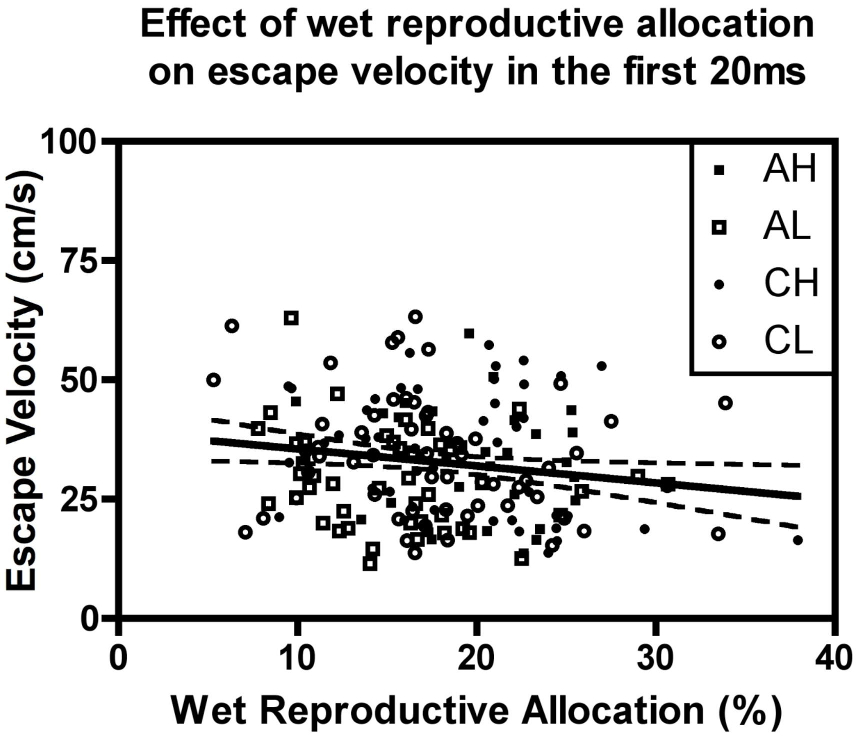


Figure 3.3. As wet RA increases, escape velocity during the first 20 ms of an escape response decreases.

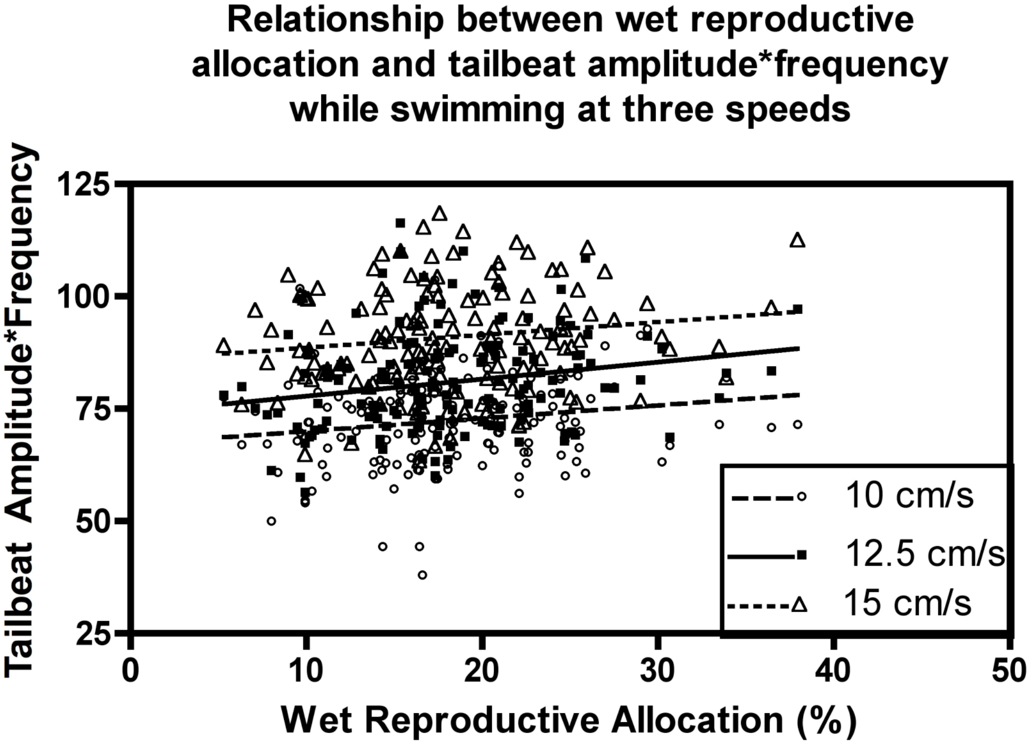


Figure 3.4. Tailbeat amplitude*frequency was larger in females with higher reproductive allocations, indicating they had to work harder to swim a fixed speed.

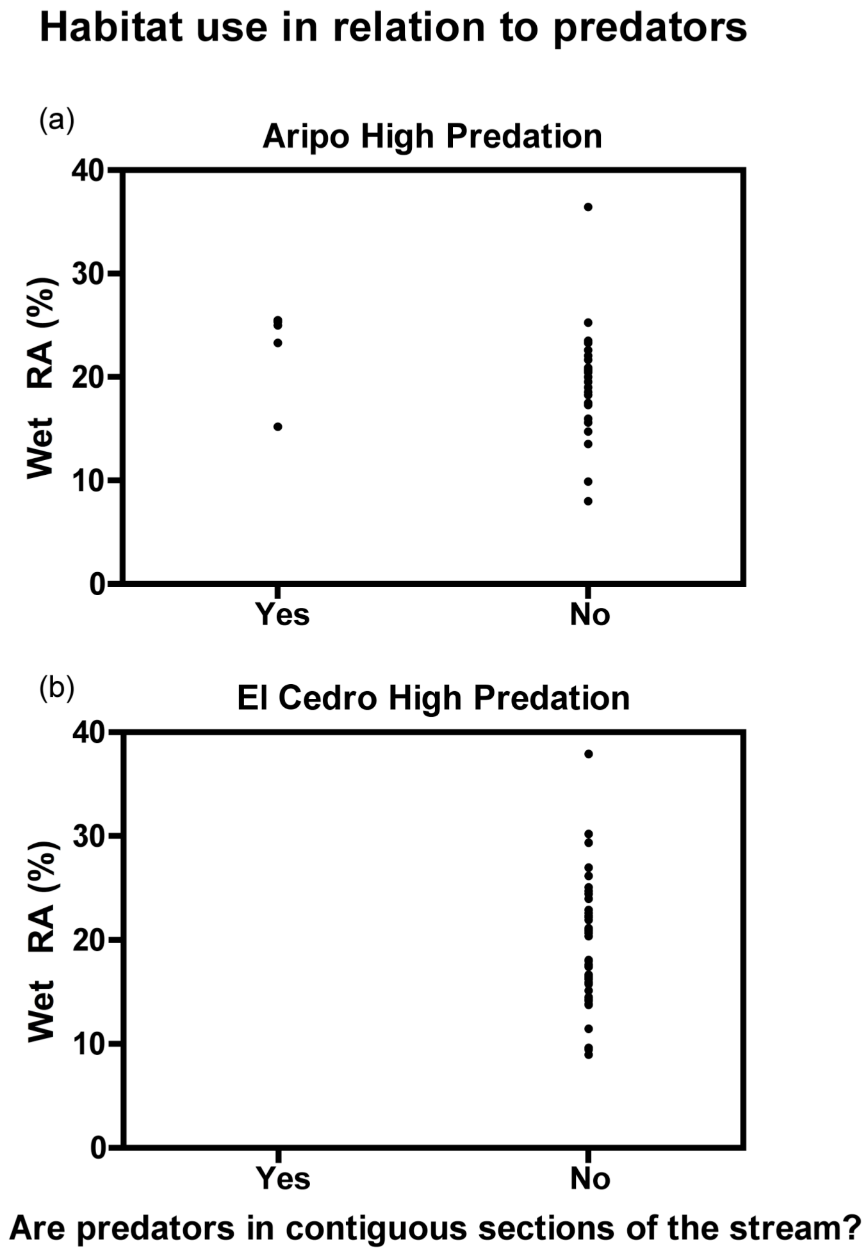


Figure 3.5. Habitat use by pregnant females in relation to predators. No relationship was found between wet RA and proximity to predators in either drainage. In the Aripo drainage, there was no significant difference between the RAs of those females found near predators and those that were not. In El Cedro drainage, no female used sections of the stream near predators, preventing analysis.

Relationship between wet reproductive allocation and habitat water flow velocity

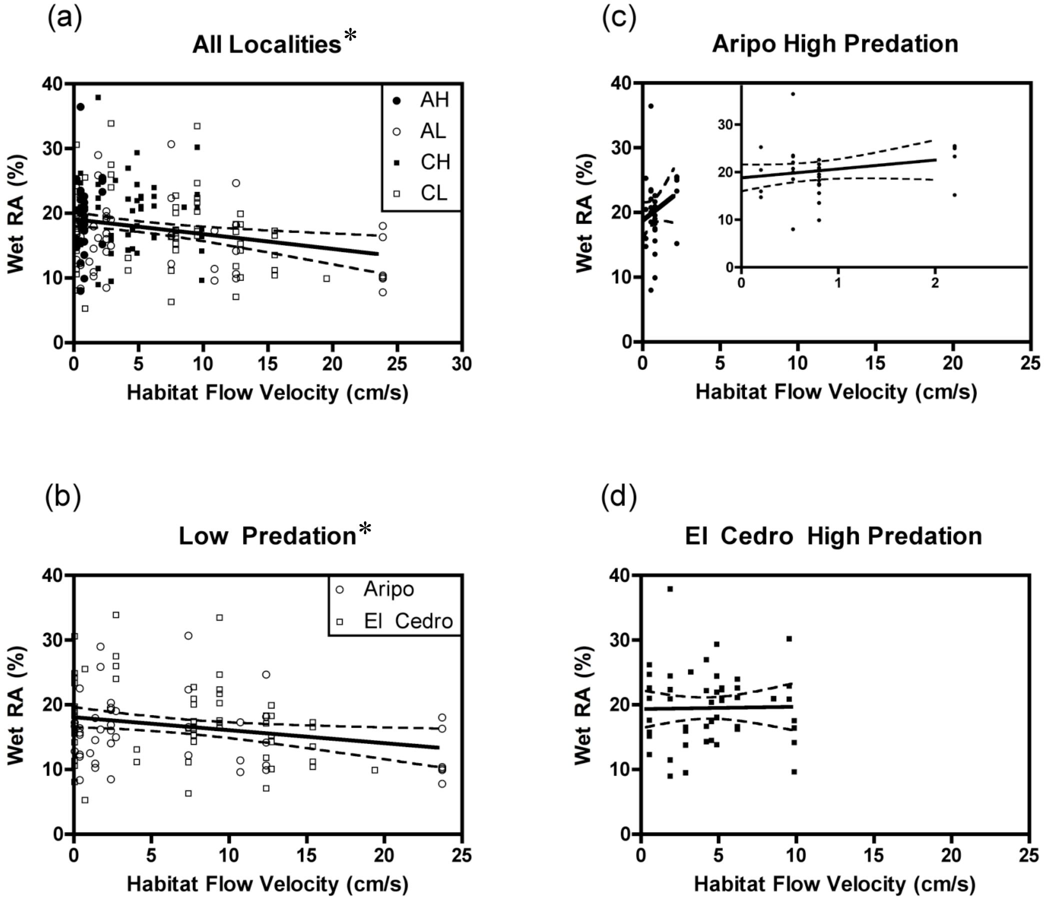


Figure 3.6. Habitat use by pregnant females in relation to water flow velocity. When all drainages were pooled, females with higher wet RAs were found in significantly lower water velocities. There were significant effects of drainage, predation, and the interaction between the two (a). Low predation populations did not respond differently, so they were pooled for analysis. Again, females with higher wet RAs were found in significantly lower water velocities (b). High predation localities modified habitat used differently between drainages so were separated for analyses. Neither drainage showed a significant relationship between wet RA and habitat water flow velocity (c&d). Inset of (c) shows detail at finer scale.

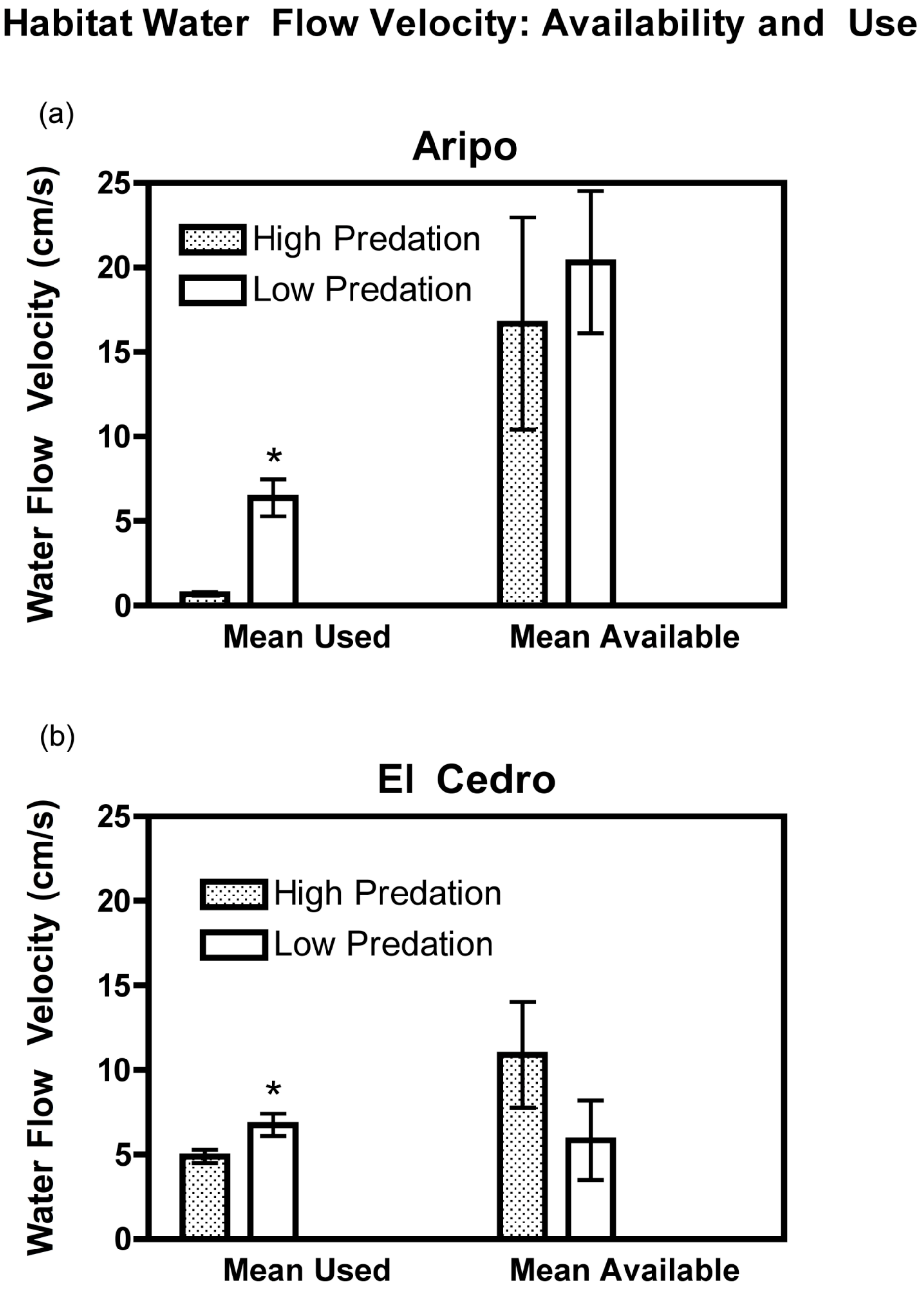


Figure 3.7. In both the Aripo (a) and El Cedro (b), high predation fish used significantly slower water flow velocities despite no significant difference in the mean water flow velocity available to them.

CONCLUSION

In this dissertation, I examined various selective factors that may contribute to the evolutionary diversification of reproductive modes within livebearers. Livebearing is thought to inflict costs that reduce fecundity and survival of a female (Shine & Bull 1979), and several studies have provided evidence to support various assumptions and predictions of this claim (Brodie 1989, Shine 1980, Shine & Bull 1979, Van Damme et al. 1989, Walker et al. 2005). The research contained in this dissertation examines subsequent evolution after a species becomes viviparous, which may reduce these associated costs.

Within livebearers, the placenta has evolved multiple independent times, indicating it may be the result of strong selection. The factors contributing to its selection, however, remain unclear. A number of hypotheses have been proposed to explain why placental matrotrophy would have evolved from lecithotrophy (Crespi & Semeniuk 2004, Haig 1993, Thibault & Schultz 1978, Trexler & DeAngelis 2003, Zeh & Zeh 1996). These hypotheses fall into two main categories. Adaptive hypotheses assume the placenta confers some sort of fitness benefit to the female, usually in terms of increased fecundity (Trexler & DeAngelis 2003), or by reducing the locomotory costs of retaining young during development (Thibault & Schultz 1978). Conflict hypotheses instead assume that the placenta itself is the result of parent-offspring conflict manifested in utero. In this scenario, the offspring attempts to elicit more resources than is in the best interest of the mother, while the mother attempts to allocate resources to offspring in such a way that will maximize her fecundity. Over time, each participant in the conflict develops a series of adaptations to counter the actions of the other, with the result being a placenta of increasing complexity (Crespi & Semeniuk 2004, Haig 1993, Haig 1996, Zeh & Zeh 1996). This main goal of this research was to focus on adaptive hypotheses for the evolution of the placenta, but along the way data collected also provided unanticipated information relevant to conflict hypotheses.

The Trexler-DeAngelis model is the only published non-verbal model for the evolution of matrotrophy (Trexler & DeAngelis 2003). The model contains several unverified assumptions and predictions, and provided an excellent starting point for empirical studies. The model is based on a well-documented difference in the biology of lecithotrophic and matrotrophic species; lecithotrophs start with large, fully yolked eggs, while matrotrophs start with small eggs and allocate nutrients throughout gestation. Because of this, matrotrophs can produce a larger brood size with a set amount of starting resources. Given enough resources over the course of gestation to provision these young, the matrotroph thus has the potential to have higher fecundity than a lecithotroph.

A key assumption of the model is that matrotrophic species can abort offspring in low food conditions. Without this ability, matrotrophy will only be favored in a narrow range of conditions where resources consistently meet or exceed energy demands of the female and her developing offspring; otherwise she risks spreading her resources too thin and losing all offspring in a developing brood. I tested this assumption in a replicated experiment using two closely related pairs of matrotrophic and lecithotrophic species from the northern and southern clades of the genus *Poeciliopsis*, each of which represent an independent origin of the placenta. Using a food manipulation experiment, I found no evidence that matrotrophs can abort offspring in low food conditions. An additional assumption of the model was that terminal offspring size is the same for both reproductive modes, irrespective of resource availability. However, my research shows that in matrotrophs, broods that were initiated before the food treatments began responded to a decrease in food level by producing either lighter or leaner offspring. Numerous studies have shown that lighter or leaner offspring have lower survival, particularly in low food conditions (Einum & Fleming 2004, Ferguson & Fox 1984, Gliwicz & Guisande 1992, Hassall et al. 2006, Hutchings 1991, Parichy & Kaplan 1992), suggesting the matrotrophic response to fluctuations in resource level may be maladaptive.

An unexpected finding from the above studies is related to resource partitioning between somatic tissues and reproduction. In both the northern and southern clade, placental females sacrificed body condition in order to maintain a consistent level of reproductive allocation when faced with low food conditions. Non-placental females instead maintain body conditions at the expense of reproductive allocation. This pattern in resource partitioning can confidently be attributed to reproductive mode rather than the suite of other life history traits because the northern and southern clades of the genus exhibit inverse relationships between the presence of the placenta and suite of other life history traits (Bassar et al., unpublished data).

Considered together, the results of these two studies point to the conflict hypothesis as an alternative explanation for the evolution of the placenta. If the mother were completely in charge of resource provisioning, I would expect her to abort a subset of the offspring in low food conditions as described in the Trexler-DeAngelis model. Instead, in low food conditions she produces low quality offspring and suffers a deterioration of somatic condition, which likely reduces her residual reproductive value. This outcome seems maladaptive for both mother and offspring, suggesting that parent-offspring conflict may be mediating this interaction. While not conclusive, this information adds to the growing body of circumstantial evidence that supports conflict hypotheses for the evolution of the placenta.

The dissertation also explores a second category of adaptive hypotheses that focus on the locomotory costs associated with pregnancy. A larger reproductive package contributes to a reduction in locomotor performance, both in terms of escape response and prolonged locomotion (Ghalambor et al. 2004, Plaut 2002). Small reductions in escape performance can lead to a decreased probability of survival during a predator attack (Walker et al. 2005). Studies that have looked for correlates between life history traits and the presence of the placenta have found that the only common correlate across taxa is a reduction in reproductive allocation (Reznick et al. 2007). Because of this, matrotrophy is hypothesized to have evolved as the result of selection for lower reproductive allocation, which would decrease the locomotory costs of pregnancy.

However, no study has shown how variation in reproductive allocation and locomotor performance translates to behavior in a natural setting. The goal of the final study was to examine the link between reproductive allocation, locomotion, and habitat use in the wild. To do this I used natural populations of Trinidadian guppies. Guppies are lecithotrophic, but show an increase in wet reproductive allocation over the course of gestation. This variation made it possible to address the costs of higher reproductive allocation and to make indirect inferences about what might be gained from being matrotrophic.

I found that escape behavior and prolonged swimming performance declined as reproductive allocation increased. In addition, females with higher reproductive allocations reduced the locomotor cost of reproduction by using habitat with lower water flow velocities, at least in low predation environments. Predators seem to restrict habitat use to a narrower range of water flow velocities, which may have obscured the effect of increased reproductive allocation on habitat water flow velocity in high predation environments. Anecdotally, habitat with faster water flow velocity is the preferred feeding ground for females. If this is true, then there are likely costs associated with the behavioral changes found in this study. Because matrotrophs consistently have a lower reproductive allocation than lecithotrophs, it may serve as a way to reduce the locomotory costs of reproduction without leaving the preferred feeding grounds.

The research contained in this dissertation does not allow us to make sweeping conclusions regarding why the placenta would have evolved. It instead contributes incremental steps in knowledge, which are vital pieces of the puzzle. Future research should focus on several areas. First, the Trexler-DeAngelis mode should be modified to more accurately reflect the biology of the reproductive modes. This modification could give us new insight into how resource availability affects placental and non-placental species differently. Second, emphasis should be given to studies that explore conflict hypotheses for the evolution for the evolution of the placenta. Crespi and Semeniuk (2003) outline a number of predictions that could be used as a starting point for investigation. Third, comparative studies should be conducted that characterize the locomotory costs (both kinematic and physiological) of pregnancy on placental and non-placental females. Thus far, locomotor studies have focused primarily on lecithotrophic animals. Studies including both extremes in the reproductive continuum can tell us whether the results from these studies are truly applicable to placental species. Fourth, experimental verification is needed to conclude whether modification of habitat use due to increased reproductive allocation restricts a female to areas with lower food quality. Finally, quantification of the hydrological profiles of the habitats where placental and non-placental fish are found is needed, in order to look for correlations between water flow velocity and reproductive mode.

REFERENCES:

Brodie, E. D. (1989). Behavioral modification as a means of reducing the cost of reproduction. *American Naturalist* **134**, 225-238.

Crespi, B. & Semeniuk, C. (2004). Parent-offspring conflict in the evolution of vertebrate reproductive mode. *American Naturalist* **163**, 635-653.

Einum, S. & Fleming, I. A. (2004). Environmental unpredictability and offspring size: conservative versus diversified bet-hedging. *Evolutionary Ecology Research* **6**, 443-455.

Ferguson, G. W. & Fox, S. F. (1984). Annual variation of survival advantage of large juvenile side-blotched lizards, Uta stansburiana: its causes and evolutionary significance. *Evolution* **38**, 342-349.

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

Gliwicz, Z. M. & Guisande, C. (1992). Family planning in Daphnia: resistance to starvation in offspring born to mothers grown at different food levels. *Oecologia* **91**, 463-467.

Haig, D. (1993). Genetic conflicts in human pregnancy. *Quarterly Review of Biology* **68:4**, 495-532.

Haig, D. (1996). Gestational drive and the green-bearded placenta. *Evolution* **93**, 6647-6551.

Hassall, M., Walters, R., Telfer, M. & Hassall, M. R. J. (2006). Why does a grasshopper have fewer, larger, offspring at its rage limits? *Journal of Evolutionary Biology* **19**, 267.

Hutchings, J. A. (1991). Fitness consequences of variation in egg size and food abundance in brook trout Salvelinus fontinalis. *Evolution* **45**, 1162-1168.

Parichy, D. M. & Kaplan, R. H. (1992). Maternal effects on offspring growth and development depend on environmental quality in the frog Bombina orientalis. *Oecologia* **91**, 579-586.

Plaut, I. (2002). Does pregnancy affect swimming speed in female mosquitofish, *Gambusia affinis? Functional Ecology* **16**, 290-295.

Reznick, D., Meredith, R. & Collette, B. B. (2007). Independent evolution of complex life history adaptations in two families of fishes, live-bearing halfbeaks (Zenarchopteridae, Beloniformes) and Poeciliidae (Cyprinodontiformes). *Evolution* **61**, 2570-2583.

Shine, R. (1980). "Costs" of reproduction in reptiles. *Oecologia* **46**, 92-100.

Shine, R. & Bull, J. (1979). The evolution of live-bearing in lizards and snakes. *American Naturalist* **113**, 905-923.

Thibault, R. & Schultz, R. J. (1978). Reproductive adaptations of viviparous fishes (*Cyprinodontifomes: Poeciliidae)*. **32:2**, 320-333.

Trexler, J. & DeAngelis, D. (2003). Resource allocation in offspring provisioning: an evaluation of the conditions favoring the evolution of matrotrophy. *American Naturalist* **162**, 574-585.

Van Damme, R., Bauwens, D. & Verheyen, R. (1989). Effect of relative clutch mass on sprint speed in the lizard *Lacerta vivipara*. *Journal of Herpetology* **23**, 459-461.

Walker, J. A., Ghalambor, C., Griset, O. L., McKenney, D. & Reznick, D. (2005). Do faster starts increase the probability of evading predators? *Functional Ecology* **19**, 808-815.

Zeh, J. & Zeh, D. (1996). The evolution of polyandry I: intragenomic conflict and genetic incompatibility. *Proceedings of the Royal Society* **263**, 1711-1717.