Life history of *Gambusia vittata* (Pisces: Poeciliidae)

Meaghan L. Weldele[1], J. Jaime Zúñiga-Vega[2*] and Jerald B. Johnson[1]
[1]Evolutionary Ecology Laboratories, Department of Biology, Brigham Young University. Provo 84602, Utah, USA.

[2]Departamento de Ecología y Recursos Naturales, Facultad de Ciencias, Universidad Nacional Autónoma de México. Ciudad Universitaria 04510, Distrito Federal, México. jzuniga@ciencias.unam.mx

**Correspondent: jzuniga@ciencias.unam.mx*

ABSTRACT--Viviparous fishes of the family Poeciliidae have been model systems in the study of life histories because of their remarkable variation in life-history traits both within and among species, and because of the diversity of selective environments that they occupy. However, the life histories of several poeciliids remain unknown. In this study, we describe the life history of *Gambusia vittata,* a poorly know poeciliid endemic to eastern Mexico. We analyze variation in number of embryos per reproductive female, size of individual embryos, size at maturity, reproductive effort, the amount of maternal transfer of nutrients to developing embryos, and the relationship between female size and life-history traits. Individuals were collected from three sites across three months. We found that brood size varied significantly across sites and that brood size, embryo mass, and reproductive allotment varied across months within sites. Brood size, embryo mass, and reproductive allotment all increased with female size. We found that this species exhibits moderate matrotrophy, meaning that females transfer small amounts of nutrients to embryos during development. We compare our results to other poeciliid species and provide tentative explanations for the patterns of intraspecific variation that we observed in the life history of *G. vittata*.

RESUMEN--Los peces vivíparos de la familia Poeciliidae han sido sistemas modelo en el estudio de historias de vida debido a la gran variación que presentan en sus atributos de historias de vida tanto dentro de como entre especies, y debido a la diversidad de ambientes selectivos que ocupan. Sin embargo, las historias de vida de muchos poecílidos permanecen desconocidas. En este estudio, describimos la historia de vida de *Gambusia vittata,* un poecílido poco conocido y endémico del este de México. Analizamos la variación en el número de embriones por hembra reproductiva, el tamaño individual de los embriones, la talla a la madurez, el esfuerzo reproductor, el nivel de transferencia materna de nutrientes a los embriones en desarrollo y la relación entre el tamaño de la hembra y las características de historia de vida. Los individuos fueron colectados de tres sitios en tres meses distintos. Encontramos que el tamaño de la camada varió significativamente entre sitios y que el tamaño de la camada, la masa de los embriones y el esfuerzo reproductor variaron entre meses dentro de los sitios. El tamaño de la camada, la masa de los embriones y el esfuerzo reproductor aumentaron con el tamaño de la hembra. Encontramos que esta especie exhibe matrotrofía moderada, lo que significa que las hembras transfieren pequeñas cantidades de nutrientes a los embriones durante el desarrollo. Comparamos

nuestros resultados con otras especies de poecílidos y presentamos explicaciones tentativas a los patrones de variación intra-específica que observamos en la historia de vida de *G. vittata.*

No other field of science gets closer to the traits that underpin fitness than the study of life histories. Life histories are related directly to selection, adaptation, speciation, and physiological constraints, which are all crucial in understanding the evolution of living things (Stearns, 1992). By comparing populations and species with different life-history traits we can also better understand the environmental factors that shape evolutionary diversification (Roff, 2002). Hence, the study of life histories offers key insights into some of our most fundamental questions in ecology and evolutionary biology.

Viviparous fishes, such as those from the family Poeciliidae, are excellent models for understanding the evolution of life cycles. There are nearly 220 species of poeciliid fish known (Pires et al., 2011). This abundance of species makes it easy to conduct comparative studies and to find links between life-history evolution and causes of natural selection (Johnson and Bagley, 2011). Life-history traits of poeciliid fishes vary not only across species, but also across populations of a single species. Studying the life histories of different populations in the same species can help us to recognize the driving forces behind the observed differences among populations in life-history traits (Johnson and Bagley, 2011). This is because within the few species that are thoroughly studied (e.g., *Poecilia reticulata, Gambusia affinis, Brachyrhaphis rhabdophora*), the species and populations evolved the same traits independently and as a response to the same environmental factors such as predation, stream velocity, or population density (Reznick and Endler, 1982; Johnson and Belk, 2001; Reznick et al., 2002; Zúñiga-Vega et al., 2007).

To gain a complete comprehension of the diversity of life cycles of viviparous fishes and of the evolutionary causes of this diversity, we must expand our knowledge to multiple species within the same family. Only a few species of poeciliid fishes have been examined in terms of spatial and temporal variation in their life histories (Johnson and Bagley, 2011). Of those examined, several have life history descriptions based on a single site or for collections made at a single point in time (e.g., Johnson, 2002; Patimar et al., 2011; Gkenas et al., 2012). However, for a complete understanding of the evolution of life histories within the family Poeciliidae we need thorough descriptions of variation among multiple populations through both space and time, with a particular emphasis on species for which no life history data are available.

In this paper we describe spatial and temporal variation in life-history traits of the viviparous fish *Gambusia vittata*, a species whose life history remains undescribed. Specifically, we analyze variation in number of embryos per reproductive female, individual embryo mass, size at maturity, reproductive effort, the amount of maternal transfer of nutrients to developing embryos, and the relationship between female size and life-history traits. We collected data from three sites over three months and tested for differences in life-history traits among sites. Two of our study sites, although separated by approximately 120 km (linear distance), were located within the same river drainage. Therefore, we predicted that the two sites located in the same river drainage would be more similar to each other, in terms of life-history traits, compared to our third site, which is located in a smaller river that has no inland contact with the other. We also tested for temporal variation in the life history of *G. vittata*. We had data for January, March, and April. These months correspond to the dry season. However, we still predicted

differences among these months. Finally, we compared the life history of *G. vittata* to other poeciliid fishes that are studied thoroughly. We discuss our findings in an ecological and evolutionary context in an attempt to understand the causes that are driving temporal and spatial variation in the life history of this species.

MATERIALS AND METHODS--*Study Species*--*Gambusia vittata* is a viviparous fish of the family Poeciliidae. In males the anal fin is modified to form a structure that is used to fertilize females (gonopodium). *Gambusia vittata* is endemic to a small region in mid-eastern Mexico. It occurs mainly in two river drainages: the Tamesí River and the Pánuco River. *Gambusia vittata* inhabits streams, canals, rivers, and ditches that are normally clear. Occasionally the currents in these water systems can be strong, but typically they are light to moderate (Miller et al., 2005).

Gambusia vittata was originally described by Hubbs (1926), based only on the morphological examination of preserved females. Later, he was able to collect males and, based on characteristics of the male gonopodium, he assigned this species to a different monotypic genus. Hubbs named the species *Flexipenis vittatus* (in Rivas, 1963: 334). According to this author, the gonopodial structures of *F. vittatus* differ substantially from those observed in other members of the genus *Gambusia* and, in contrast, resemble those of males of the genera *Belonesox* and *Heterophallus*. Rauchenberger (1989) reinstated this species within *Gambusia,* because her detailed morphological examination revealed that *G. vittata* exhibits the synapomorphies that she described for the genus *Gambusia*. Rauchenberger (1989) interpreted the distinct gonopodium of *G. vittata* as retention of a primitive character. Phylogenetic analyses based on genetic data confirmed the current taxonomic status of *G. vittata* (Lydeard et al., 1995; Hrbek et al., 2007).

Little is known of the biology of *G. vittata*. Darnell (1962) examined the stomach contents of several specimens of *G. vittata* and found that 50% of the material encountered was filamentous algae. Only 20% of the stomach contents consisted of arthropods. The remaining 30% consisted of detritus and undetermined organic matter. These feeding habits are unusual compared to other congeneric species, which feed predominantly on arthropods (Darnell, 1962). Apparently, females of *G. vittata* undergo a period of fasting when embryos approach full term. After the young are born, females feed heavily on algae, presumably to avoid eating their own offspring (Darnell, 1962). A description of the external female genitalia of *G. vittata* was provided by Peden (1972). According to this author, females of *G. vittata* exhibit the most distinct and differentiated genitalia of all *Gambusia* species (similar to the male gonopodium of *G. vittata*). Males mate with females by means of gonopodial thrusts, with no apparent courtship behavior (Bisazza, 1993). The karyotype of this species consists of 48 (2n) non-metacentric chromosomes (Campos and Hubbs, 1971). A description of helminth parasites present in *G. vittata* has been provided by Salgado-Maldonado et al. (2004). The exotic and highly pathogenic tapeworm *Bothriocephalus acheilognathi* has been collected from the intestine of *G. vittata* specimens (Salgado-Maldonado and Pineda-López, 2003).

Study Sites and Field Methods--Individuals of *G. vittata* were collected from three different sites in the mid-eastern region of Mexico (Fig. 1). Table 1 shows a detailed description of the three study sites, including a list of other fish species present. We used seines (1.3 m depth × 5 m length, 8 mm mesh) to capture individuals. Following capture, each fish was euthanized in 3-amenobenzoic acid ethyl ester (MS-222). Rather than preserving the fish in formalin, specimens were fixed in the field in 95% ethanol and stored in the laboratory in 70% ethanol.

This allowed us to preserve samples so that they could subsequently be used for DNA sequencing.

We collected *G. vittata* individuals from three sites across three months during the dry season. Our first collections were in January 2012 from all three sites. Though we collected females in January from site 3, only two females contained embryos. Therefore, we removed these two females from all analyses. We collected again from all three sites in April 2012. In March 2013 we were able to collect again from site 2 only. The chronological order we collected specimens (the order the data are shown throughout the paper) is January 2012, April 2012, and March 2013. Site 2 is the only site with data collected for the month of March (refer to Table 1 for detailed descriptions).

Quantifying Life-History Traits--We quantified five life-history traits for each population each month: (1) number of embryos per reproductive female (brood size), (2) individual embryo mass (dry mass), (3) size at maturity for both males and females, (4) reproductive allotment (RA), and (5) the amount of maternal transfer of nutrients to developing embryos (i.e., degree of matrotrophy). All data were collected by dissecting preserved females, with one exception: preserved males were used to estimate male size at maturity. Brood size was determined by counting the number of embryos each female contained. The stage of development was identified following Haynes (1995). Individual embryo mass was estimated as the average dry weight of embryos from each female. To estimate female size at maturity, we classified all our females into 2-mm size classes, based on their standard length (SL). The minimum size at maturity corresponded to the size class in which at least 50% of the females contained embryos or vitellogenic follicles (Reznick and Endler, 1982; Johnson and Belk, 2001). Previous studies have estimated male size at maturity as the average SL of adult males, because apparently males cease growing upon maturity (Johnson and Belk, 2001; Jennions et al., 2006; Scott and Johnson, 2010). However, in *G. vittata* we have observed wide variation in the sizes of mature males (15 – 26 mm SL). Therefore, we estimated the minimum size at maturity for males following a similar procedure as females. We classified all males into 2-mm size classes. To estimate male size at maturity, we examined the development of the gonopodium. The minimum size at maturity corresponded to the size class in which at least 50% of the males exhibited completely developed gonopodia. We calculated the reproductive allotment (RA) of each female as the total dry weight of the brood. We also measured the dry mass of reproductive females. Dry masses for all females and embryos were measured after desiccation in an oven for 24 hours at 55 ºC.

The amount of nutrient transfer to developing embryos was estimated using the matrotrophy index (MI) (Reznick et al., 2002; Thompson et al., 2002). We calculated an MI for each population in each month. The MI was calculated by dividing the estimated dry mass of the embryos near time of birth (stage 11 according to Haynes, 1995) by the estimated dry mass of the embryos at the time of fertilization (stage 4). We estimated these dry masses using a regression between stage of development as the independent variable and embryo mass (log-transformed) as the dependent variable for each site and for each month.

Researchers have characterized maternal provisioning of embryos using three categories: matrotrophic, lecithotrophic, and incipient matrotrophic (Blackburn, 1992; Reznick et al., 2002; Thompson et al., 2002). Matrotrophic organisms provide considerable amounts of nutrients to their developing embryos. Lecithotrophic organisms provide all nutrients necessary for development before fertilization in the form of yolk. Incipient matrotrophic organisms transfer small quantities of nutrients to developing embryos to offset some of the metabolic costs. In order to be considered matrotrophic, the MI for a population must range between 1.0, meaning

that a small amount of nutrients was transferred to the embryos after fertilization, and >100, which implies that a large amount of nutrients was given to the embryos post-fertilization. Lecithotrophic populations will have an MI of around 0.6 - 0.7, values consistent with the mass lost by the embryos during development due to metabolic expenses and lack of female nutrient transfer. Finally, if a population has an MI between 0.7 - 1.0 then they are considered incipient matrotrophic. This means that they will lose less mass during development compared to lecithotrophic embryos because they have a small amount of nutrient transfer from their mothers during development (Reznick et al., 2002; Thompson et al., 2002).

Statistical Analyses--To compare brood size, individual embryo mass, and RA among populations and between months we used general linear models (GLM). We conducted one linear model for each of these three traits. In all three analyses, female dry mass was used as a covariate and both source population and sampling month (January 2012, April 2012, or March 2013) were used as categorical factors. The factor "sampling month" was nested in the factor "population". We used stage of development as an additional covariate when analyzing individual embryo mass. We log-transformed individual embryo mass, RA, and female mass to meet the assumptions of normality and variance homogeneity. Brood size was square-root transformed. To facilitate interpretation and visualization of the GLM results, we present figures and least-square means obtained from similar analyses conducted on untransformed data.

To compare the degree of maternal transfer of nutrients to developing embryos, we constructed 95% confidence intervals for our MI values by means of a resampling procedure (as per Zúñiga-Vega et al., 2011). First, for each population and sampling month we bootstrapped our individuals to obtain a new sample. Second, based on this new sample, we fitted a regression between stage of development and embryo mass. Third, from this regression we estimated embryo mass at fertilization (stage 4) and embryo mass at birth (stage 11) and based on these estimates we calculated a new MI. We repeated this procedure 1000 times generating 1000 MI values for each month and population. To construct 95% confidence intervals, we used the 25th and 975th sorted values of the resulting distribution of MI values as the lower and upper limits, respectively.

RESULTS--The average size of mature females across all sites and months was 22.2 mm SL while the average size of mature males across all sites and months was 20.8 mm SL. The average dry mass of mature females (excluding digestive tract and embryos) across all sites and months was 0.049 g. Considering only females of adult size (i.e., larger than the minimum size at maturity), the proportion of reproductive females varied from around 0.77 to 1.0 across sites and months (Fig. 2). The largest difference among months occurred at site 1. In January 2012, 77.8% of adult-sized females had developing embryos. In contrast, in April 2012 a larger percentage of adult-sized females (98.3%) contained developing embryos (Fig. 2a). At site 2, the proportions of reproducing females were similar in January 2012 (0.83) and March 2013 (0.79), but at this site again in April 2012, we found a higher proportion of adult-sized females that were reproductive (0.96) (Fig. 2b). At site 3 in April 2012, we found that all adult-sized females contained developing embryos (Fig. 2c). Although not included in our analyses, we noted that the two adult females collected from site 3 in January 2012 contained developing embryos (Fig. 2c).

We found significant differences among months for brood size ($F_{3, 172} = 3.42$, $P = 0.019$; Table 2). Within sites, brood size increased in March/April compared to January (Fig. 3a). Significant variation in brood size was present across sites as well ($F_{2, 172} = 4.06$, $P = 0.019$;

Table 2). The largest mean brood size was observed at site 1 (adjusted least-square mean = 5.62 young), whereas the smallest was observed at site 2 (adjusted least-square mean = 4.88 young) (Table 3). There was also a significant effect of female mass on brood size ($F_{1, 172} = 325.93$, $P < 0.0001$; Table 2). Heavier (larger) females produced larger broods (Fig. 4a). The interaction between site and female mass significantly affected brood size ($F_{2, 172} = 4.03$, $P = 0.02$). In spite of this significant slope heterogeneity, the least-square means derived from this model were comparable among sites because the slopes crossed outside of the biologically meaningful values of female mass.

Significant differences in embryo size occurred among months ($F_{3,169} = 3.67$, $P = 0.014$; Table 2). For instance, embryo mass at site 2 decreased in April 2012 compared to January 2012 and March 2013 (Fig. 3b). In contrast, there were no significant differences in embryo mass across sites ($F_{2, 169} = 0.48$, $P = 0.62$; Table 2). Mean embryo mass (adjusted least-square means) ranged between 0.0013 g and 0.0018 g (Table 3). However, this inter-site variation was not statistically significant. Female mass had a significant effect on individual embryo mass ($F_{1, 169} = 24.02$, $P < 0.0001$; Table 2). Larger females produced larger embryos (Fig. 4b). However, the effect was weaker on this trait than that observed on brood size and RA (Figs. 2a, c). Stage did not affect embryo size ($F_{1, 169} = 0.61$, $P = 0.43$; Table 2). This indicates that the embryos stay at a constant mass during development.

We found significant differences in RA among months within sites ($F_{3, 172} = 6.17$, $P = 0.0005$; Table 2). At site 1, RA during April 2012 was significantly higher than RA during January 2012. At site 2, RA increased in March 2013 compared to January 2012 and April 2012 (Fig. 3c). In contrast, no significant differences in RA were found across sites ($F_{2, 172} = 0.099$, $P = 0.91$). Mean size-adjusted RA varied form 0.0070 g in site 1 to 0.0099 g in site 3 (Table 3). Female mass had a significant effect on RA as well ($F_{1, 172} = 234.96$, $P < 0.0001$; Table 2). Larger females exhibited greater RA (Fig. 4c).

Female size at maturity varied across sites. At sites 1 and 3 females matured on average at a larger size (21 mm SL) compared to site 2 (17 mm SL) (Table 3). Our monthly variation data within sites was limited. Females collected in April 2012 from site 1 did not result in an estimate of female size at maturity because we did not find small-sized females. Similarly, females collected in January 2012 from site 3 only included two reproducing females (14 females were collected only 2 of which were reproductive). Thus, no estimate of minimum size at maturity could be obtained for January at site 3. At site 2 our monthly estimates of female size at maturity were equal among months (17 mm SL in all three months) (Fig. 3d).

Male size at maturity also varied across sites. Similar to our results for female size at maturity, at sites 1 and 3 males matured on average at larger sizes (20 and 21 mm SL, respectively) compared to site 2 (15.7 mm SL) (Table 3). We found slight variation among months within sites. At site 1 minimum size of mature males was 19 mm SL for January 2012 and 21 mm SL for April 2012 (Fig. 3e). At site 2, the smallest sizes at maturity were observed in both January 2012 and March 2013 (15 mm SL), whereas the largest for this site was observed in April 2012 (17 mm SL) (Fig. 3e). Males collected in January 2012 from site 3 did not result in an estimate of male size at maturity because we only collected three males and all of them were large-sized (>24 mm SL) and mature. Therefore, for site 3 we estimated male site at maturity only for April 2012 (21 mm SL).

We found minimal variation in MI values across sites (Table 3). Site MI values ranged between 1.06 for site 3 and 1.63 for site 1. These MI values per site were not significantly different as indicated by their overlapping confidence intervals (Table 3). This result was

consistent with the non-significant interaction between site and stage of development affecting embryo mass ($F_{2, 169} = 1.77$, $P = 0.17$; Table 2), which indicated that the relationship between embryo mass and developmental stage (i.e., the rate of mass change of embryos during development) was similar among sites. Similarly, among months within sites all the confidence intervals for the MI values overlapped, indicating the lack of significant differences (Fig. 3f). These monthly MI values ranged between 0.91 in site 2 during January 2012 and 3.96 in site 1 during January 2012.

DISCUSSION--In this study we provide the first description of the life history traits of *Gambusia vittata*, a poeciliid fish endemic to a small region in eastern Mexico. We document variation among sites and among months within sites for reproductive features. Notice that our sampling scheme included monthly variation as well as yearly variation because our samples from January and April corresponded to 2012 whereas our samples from March corresponded to 2013. For this study we assumed that variation among months was greater than variation between years and, therefore, we interpret our monthly differences as variation as the dry season progresses regardless of any particular year. However, we recognize that our results for March 2013 might include a year effect, and hence we suggest a cautious interpretation.

The proportion of reproductive females was above 0.50 for all months in all sites, which indicates that *G. vitatta* are reproductive at least from January to April. April 2012 had the largest proportion of reproductive females across all sites and months, which suggests an increase in the number of reproductive females from January to April. In most of the Mexican territory, April corresponds with the late phases of the dry season (Garcia, 2004). Therefore, our data suggest that before the beginning of the rainy season more *G. vittata* females produce offspring. Studies have found that during the wet season there is a higher abundance of resources for the young and higher water volume causing predator and prey distributions to be more regular (Winemiller, 1993; Machado et al., 2002). Similar studies have concluded that females may possess ecological and physiological sensors allowing them to sense changes in the environment (such as the beginning of the rainy season) coming on. In response to these senses, females reproduce at higher rates in order to give their offspring a higher chance of survival (Kusano, 1982; Winemiller, 1993). Therefore, the increase in proportion of reproductive females may be due to the beginning of the rainy season and associated physiological drivers.

Brood size differed among sites and among months within sites. At sites 1 and 2, brood size increased from January to April. This increase may also contribute to higher rates of reproduction just before the rainy season. The mean brood size for *G. vittata* ranged from 4.88 to 5.62 across sites (Table 3). Compared to other *Gambusia* species of similar sizes, the brood size of *G. vittata* is small. Most other *Gambusia* species have an average brood size of 10 or more offspring, which is twice as large as *G. vittata* brood sizes (e.g., *G. puncticulata puncticulata* [range of brood size across sites] = 1-70, Abney and Rakocinski, 2004; *G. affinis* [average number of embryos per brood] = 34.48, Swenton and Kodric-Brown, 2012; *G. holbrooki*, = 50.8, Gkenas et al., 2012; *G. sexradia* = 17.34, Riesch et al., 2010). An exception is *G. hubbsi*, species that exhibits a similar brood size compared to *G. vittata* (variation across low and high predation environments = 3.43 – 7.26, Riesch et al., 2013). However, when compared to other poeciliid species of similar sizes, *G. vittata* has a relatively average brood size. Several other poeciliid fishes have brood sizes ranging from around two to nine offspring (*Brachyrhaphis episcopi* = 2.9 – 8.74, Jennions et al., 2006; *Poeciliopsis prolifica* = 4.2, Pires et al., 2007; *Poeciliopsis baenschi* = 2.7, Scott and Johnson, 2010; *Heterophallus milleri* = 8.49, Riesch et al., 2011).

Statistically significant differences in brood size were found across sites. Females at sites 1 and 3 have larger broods (5.62 and 5.19 newborns), on average, than their counterparts at site 2 (4.88 newborns). This result is contrary to what was expected. Although sites 1 and 2 are in the same river drainage system, site 3 is more similar in brood size to site 1 than is site 2. This lower brood size at site 2 might represent an adaptation or a plastic response to local conditions. For instance, low resource (food) availability at site 2 might constrain the number of newborns that females can produce.

We emphasize that all the females that we dissected contained a single brood of developing embryos, indicating the lack of superfetation in this species. Superfetation is the ability of a female to simultaneously carry several broods at different developmental stages (Turner, 1937), and is present in several members of the family Poeciliidae (Reznick and Miles, 1989; Pires et al., 2011). The lack of superfetation in *G. vittata* is consistent with other *Gambusia* species (Reznick and Miles, 1989; Pires et al., 2011).

Mean individual embryo mass varied from 0.0013 to 0.0018 g across sites (Table 3). However, these differences in individual embryo mass across sites were not statistically significant. This lack of variation in embryo mass across sites is consistent with other poeciliid species (e.g., *Poeciliopsis baenschi*, Scott and Johnson, 2010; *Poecilia butleri*, Zúñiga-Vega et al., 2011). Compared to other *Gambusia* species (*G. p. puncticulata* = 0.00129 – 0.005 g, Abney and Rakocinski, 2004; *G. sexradiata* [mean embryo mass] = 0.0014 g, Riesch et al., 2010; *G. hubbsi* [across low and high predation environments] = 0.0013 – 0.0046 g, Riesch et al., 2013) the individual embryo mass for *G. vittata* is relatively similar. However, when compared to the individual embryo mass of a population of *G. eurystoma* living in a sulfidic habitat (0.0064 g, Riesch et al., 2010) the embryo mass of *G. vittata* is significantly smaller. When compared to other poeciliid species of about the same size, *G. vittata* has a similar individual embryo mass (*Heterophallus milleri* [average embryo mass] = 0.0012 g, Riesch et al., 2011; *Poecilia butleri* [range of variation across populations] = 0.0012 – 0.0021, Zúñiga-Vega et al., 2011).

Stage of development did not affect embryo mass. The mass of individual embryos remains constant throughout development. This indicates that *G. vittata* is slightly matrotrophic. Therefore, females transfer a small amount of nutrients to the developing embryos post-fertilization to offset the loss of mass experienced during development due to metabolic costs (Reznick et al., 2002; Thompson et al., 2002). The MI values that we calculated also indicate moderate matrotrophy in *G. vittata* because all of them were equal to or slightly larger than unity. This indicates a relatively similar embryo weight between fertilization and birth. The mean MI values varied little across sites (1.06 – 1.63) and only that for site 1 (1.63) was significantly higher than unity (Table 3), evidencing a slight increase in mass from fertilization to birth. With respect to monthly variation, the MI values ranged between 0.91 and 3.96. However, all these values were not statistically different from each other and most of them were not significantly different than unity, indicating relatively constant embryo mass throughout development in all our studied populations and across all the studied months. The non-significant interaction between site and stage of development affecting individual embryo mass also indicates that the mass of developing embryos remains relatively constant throughout development in all three sites.

Gambusia taxa were not expected to exhibit matrotrophy (Reznick and Miles, 1989). However, more recent studies using tritiated leucine (Marsh-Matthews et al., 2005) found that members of the *Gambusia* clade do exhibit some amount of post-fertilization nutrient transfer from mothers to embryos. Some of these species include *G. affinis* (DeMarais and Oldis, 2005;

Marsh-Matthews et al., 2005), *G. holbrooki* (Edwards et al., 2006; Marsh-Matthews et al., 2010), *G. clarkhubbsi, G. gaigei, G. geiseri, G. nobilis* (Marsh-Matthews et al., 2010), and *G. p. puncticulata* (Abney and Rakocinski, 2004). We conclude that *G. vittata* is a matrotrophic species as well. We calculated two variables that support this conclusion. First, all calculated MI values for site and month were statistically equal to or greater than 1. Second, we found no significant effect of stage of development on embryo mass. Several other poeciliid species also have MI values that indicate small amounts of maternal transfer of nutrient to embryos just as we observed in *G. vittata* (*P. latidens* = 0.86, *P. lucida* = 1.34, *P. occidentalis* = 1.12, Reznick et al., 2002; P. *butleri* = 1.07 – 5.84, Zúñiga-Vega et al., 2011). Also, the lack of significant variation in MI values across sites that we observed in *G. vittata* is congruent with studies of other poeciliid species (e.g., *P. butleri,* Zúñiga-Vega et al., 2011). This lack of variation suggests that, in some poeciliid species the degree of matrotrophy is not a plastic trait and that within these species there is no genetic variation in the degree of maternal transfer of nutrients to embryos (but see Pires et al., 2007).

Reproductive allotment did not vary significantly across sites. However, it did vary significantly across months (Table 2). In both sites 1 and 2, there was an increase in RA from January to April. This trend supports our hypothesis for an increase in reproductive effort right before the rainy season presumably for increased survival of offspring during the months with higher food availability and less density of predators and competitors (Winemiller, 1993; Machado et al., 2002). The RA also increased with female size. This is a trend that is common in other poecilliid fishes as well (Jennions et al., 2006; Johnson and Belk, 2001; Zúñiga-Vega et al., 2011). The mean size-adjusted values per site for RA of *G. vittata* varied from 0.0070 to 0.0099 g (Table 3). These values represent between 12.5% and 16.8% of the total dry mass of females. Other *Gambusia* species exhibit similar RA (*G. eurystoma* = 15.63%, Riesch et al., 2010; *G. hubbsi* [range of variation across populations] = 10.19% – 15.16%, Riesch et al., 2013). However, *G. sexradiata* exhibits more extreme RA values depending on the particular environment it inhabits. In a relatively toxic environment, *G. sexradiata* exhibits low RA (10.46%), whereas in a nontoxic environment its RA value is higher compared to *G. vittata* and other *Gambusia* species (23.03%, Riesch et al., 2010). Other poeciliid species of similar sizes show wide variation in RA. Compared to *G. vittata,* some species appear to invest less effort into reproduction (*Poeciliopsis baenschi* [range of variation across populations] = 0.0027 - 0.0044 g; Scott and Johnson, 2010), whereas females from other species make larger investments in reproduction (*Brachyrhaphis parismina* = 0.015 – 0.018 g, Belk et al., 2011; *Heterophallus milleri* = 18.87% of the total female dry mass, Riesch et al., 2011).

Females matured between 17 and 21 mm SL across all sites. Males matured between 15.7 and 21 mm SL across all sites. Females of *G. vittata* matured at similar sizes compared to other *Gambusia* species (*G. affinis* [range of variation across sites] = 16 – 26 mm SL, Stockwell and Vinyard, 2000; *G. holbrooki* = 20.35 mm SL, Gkenas et al., 2012; *G. sexradiata* = 24.19 mm SL, Riesch et al., 2010). Males of *G. vittata* also matured at similar sizes to those of other *Gambusia* species (e.g., *G. holbrooki* = 16.44 mm SL, Gkenas et al., 2012). At site 2 both males and females matured at smaller sizes, and presumably earlier, than their counterparts at sites 1 and 3. This difference is worth noting because sites 1 and 2 are in the same river drainage system. However, sites 1 and 3 are more similar to each other than are sites 1 and 2 for size at maturity. This may represent an adaptation or plastic response to local environmental conditions. Earlier studies found that predation had a large effect on life-history traits. In environments with high predation and high adult mortality rates, decreased size at maturity is favored because rapidly

maturing individuals will be more likely to reproduce prior to death (Reznick and Endler, 1982; Reznick et al., 1990; Roff, 1992; Johnson and Belk, 2001). Thus, a likely explanation for the differences that we observed in size at maturity between populations that inhabit in the same river drainage (sites 1 and 2) is the occurrence of high predator-driven adult mortality in site 2. Future demographic and genetic studies on different populations of *G. vittata* would improve our understanding of the intraspecific patterns of life-history variation that we documented here for this species.

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TABLE 1--Description of the sampling locations for *Gambusia vittata*. Values for pH, temperature, and salinity correspond to average values across sampling months. The site numbers are used throughout the paper for identification purposes.

Site number	Geographical coordinates	Altitude (m)	State	Collecting months / Sample size (males; females)	pH	Temperature (ºC)	Salinity (ppt)	Other species present	Descriptive notes
1	N 21˚ 14’ 2.2” W 98˚ 52’ 35.1”	52.8	San Luis Potosi	January 2012 / 63 (16; 47) April 2012 / 70 (9; 61)	8.04	29.2	0.48	*Poecilia mexicana*[a] *Pseudoxiphophorus jonesii*[a] *Astyanax mexicanus*[b] *Herichthys pantostictus*[c]	River with clear water and fast current. Substrate of rocks and gravel. Abundant algae. Depths to 0.7 m.
2	N 21˚ 16’ 41.8” W 97˚ 57’ 18.1”	157.4	Veracruz	January 2012 / 52 (24; 28) April 2012 / 61 (6; 55) March 2013 / 74 (42: 32)	7.61	28.2	0.58	*Poecilia mexicana*[a] *Poeciliopsis gracilis*[a] *Pseudoxiphophorus jonesii*[a] *Xiphophorus birchmanni*[a]	River with turbid water. Currents are slight to moderate. Muddy substrate. Abundant riparian vegetation and water hyacinth. Depths to 0.9 m.

									Xiphophorus nezahualcoyotl[a] *Xiphophorus variatus*[a] *Astyanax mexicanus*[b]	
3	N 21˚ 19’ 45” W 97˚ 44’ 51.2”	93.6	Veracruz	January 2012 / 17 (3; 14) April 2012 / 61 (19; 42)	7.73	24.9	0.45	*Gambusia panuco*[a] *Poecilia mexicana*[a] *Xiphophorus variatus*[a] *Astyanax mexicanus*[b] *Herichthys pantostictus*[c]	River with clear water and moderate current. Substrate of mud and bedrock. Abundant algae and water hyacinth. Depths to 0.3 m.

[a] Poeciliidae

[b] Characidae

[c] Cichlidae

TABLE 2--Results of ANCOVA model that examined number of embryos, individual embryo mass, and reproductive allotment of *Gambusia vittata*.

Life-history trait	Effect	SS	d.f.	MS	*F*	*P*
Brood Size						
	Female mass	40.26	1	40.26	325.93	< 0.0001
	Month(site)	1.27	3	0.42	3.42	0.019
	Site	1.00	2	0.50	4.06	0.019
	Site × female mass	1.0	2	0.50	4.03	0.020
	Error	21.25	172	0.1235		
Individual Embryo Mass						
	Female mass	2.91	1	2.91	24.02	< 0.0001
	Stage of embryos	0.07	1	0.07	0.61	0.43
	Month(site)	1.33	3	0.44	3.67	0.014
	Site	0.12	2	0.06	0.48	0.62
	Site × stage of development	0.43	2	0.21	1.77	0.17
	Site × female mass	0.21	2	0.10	0.86	0.42
	Error	20.48	169	0.12		
Reproductive						

Allotment						
	Female mass	47.40	1	47.40	234.96	<0.0001
	Month(site)	3.73	3	1.24	6.17	0.0005
	Site	0.04	2	0.02	0.099	0.91
	Site × female mass	0.03	2	0.02	0.08	0.92
	Error	34.70	172	0.20		

TABLE 3--Descriptive life-history traits for *Gambusia vittata*. Brood size, embryo mass, and reproductive allotment values are adjusted least square means from general linear models. Size at maturity and matrotrophy index are means from untransformed data. Values are means (S.E). For matrotrophy indexes, 95% bootstrap CIs are in parenthesis.

Site	Brood Size	Individual Embryo Mass (g)	RA (g)	Female Size at Maturity (mmSL)	Male Size at Maturity (mmSL)	MI
1	5.62 (0.25)	0.0013 (0.0001)	0.007 (0.0005)	21	20	1.63 (1.05 -2.47)
2	4.88 (0.32)	0.0017 (0.0001)	0.0081(0.0006)	17	15.7	1.17 (0.80 - 1.61)
3	5.19 (0.33)	0.0018 (0.0001)	0.0099(0.0006)	21	21	1.06 (0.77 - 1.18)

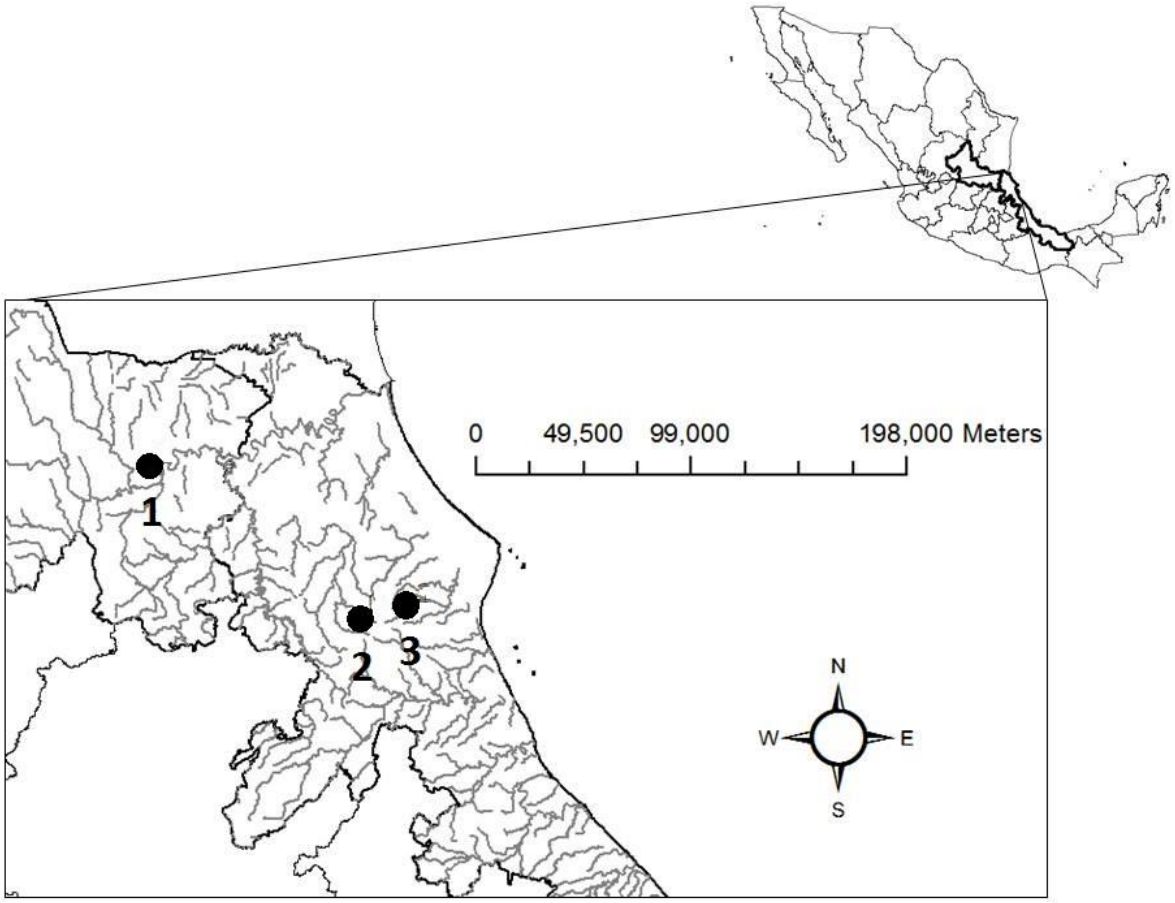
Figure legends

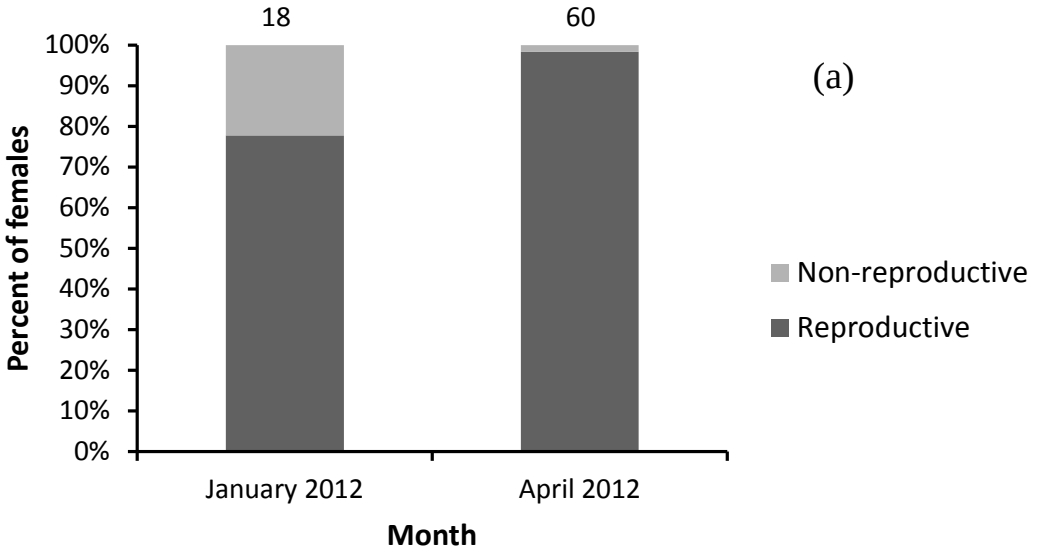
FIG. 1--Geographic location of the three populations of *Gambusia vittata*.

FIG. 2--Proportion of reproductive and non-reproductive adult-sized females of *Gambusia vittata* for site 1 (a), site 2 (b), and site 3 (c). The total number of females equal to and larger than minimum size at maturity (sample size) is shown above the corresponding sampling month.

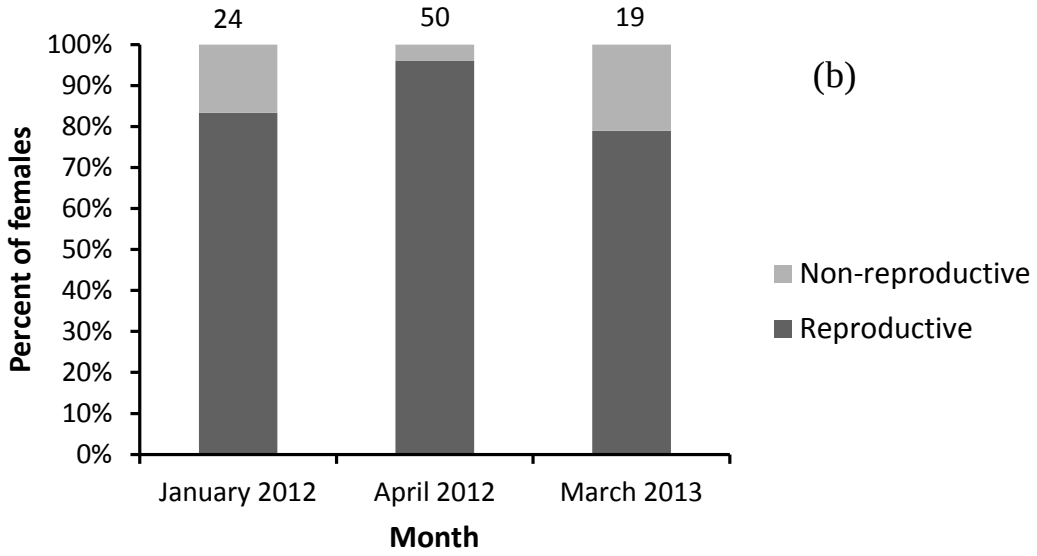
FIG. 3--Spatial and temporal variation in life-history traits of *Gambusia vittata*: total number of embryos (a), individual weight of embryos (b), reproductive allotment (c), female (d) and male (e) size at maturity, and matrotrophy index (f).

FIG. 4--Effect of female mass on life-history traits of *Gambusia vittata*: brood size (a), individual embryo weight (b), and reproductive allotment (c).

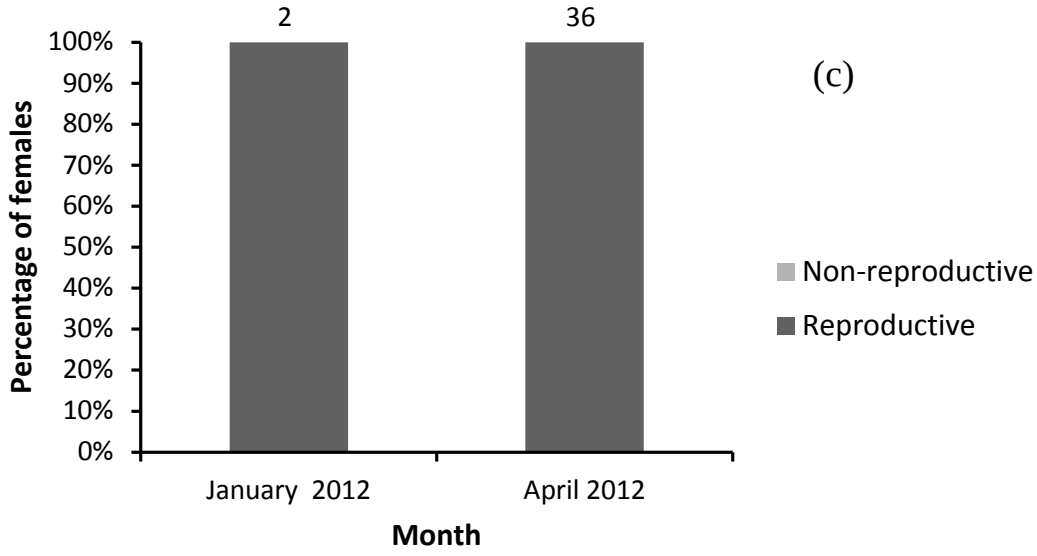

0 49,500 99,000 198,000 Meters
1
2
3
N
W
E
S


18
60
100%
90%
80%
70%
60%
50%
40%
30%
20%
10%
0%
Percent of females
January 2012
April 2012
Month
Non-reproductive
Reproductive

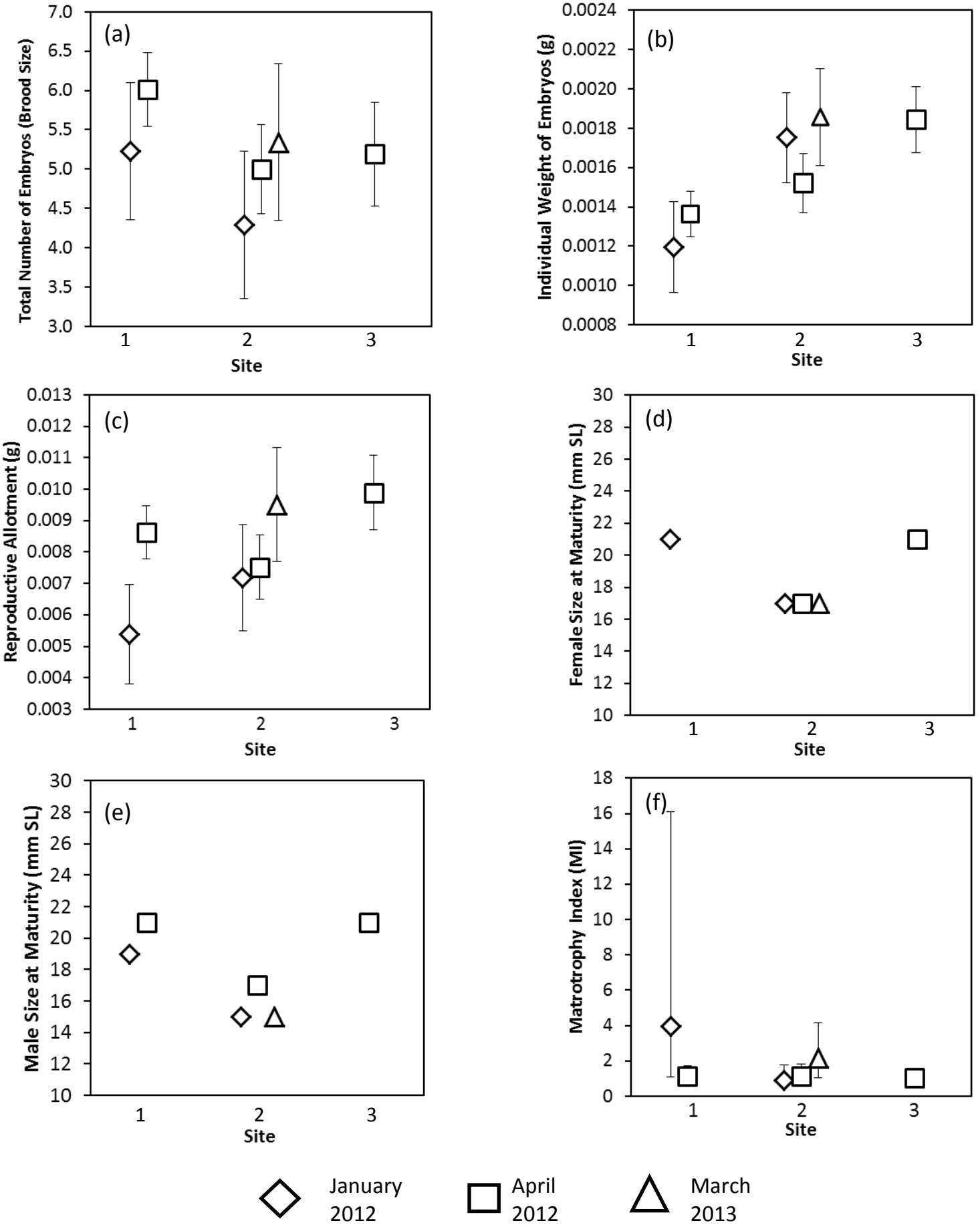
(a)

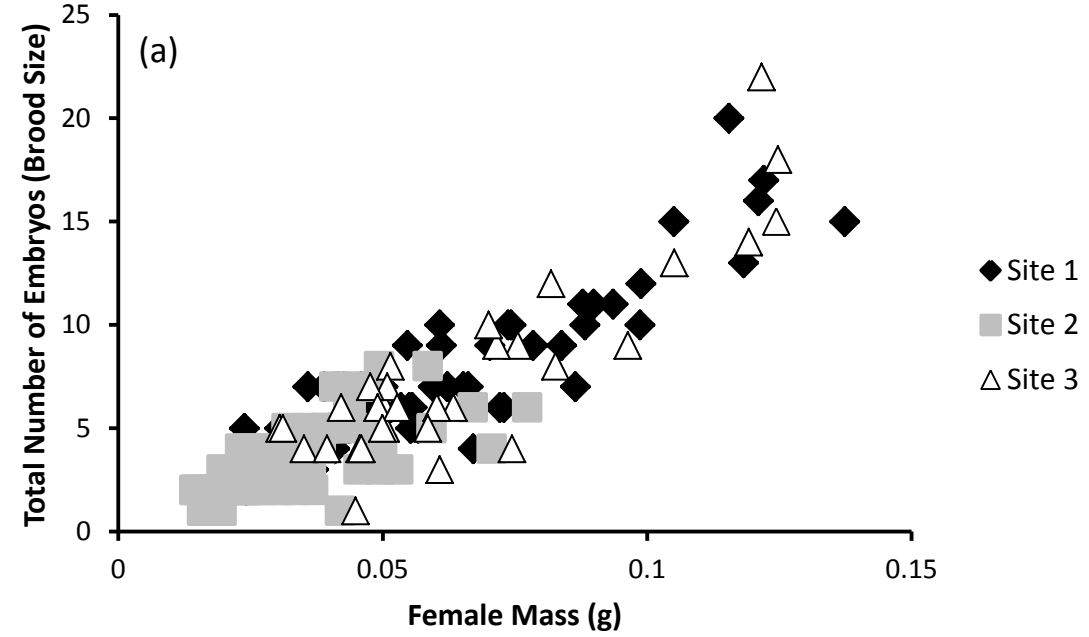

24
50
19
100%
90%
80%
70%
60%
50%
40%
30%
20%
10%
0%
Percent of females
January 2012
April 2012
March 2013
Month
Non-reproductive
Reproductive

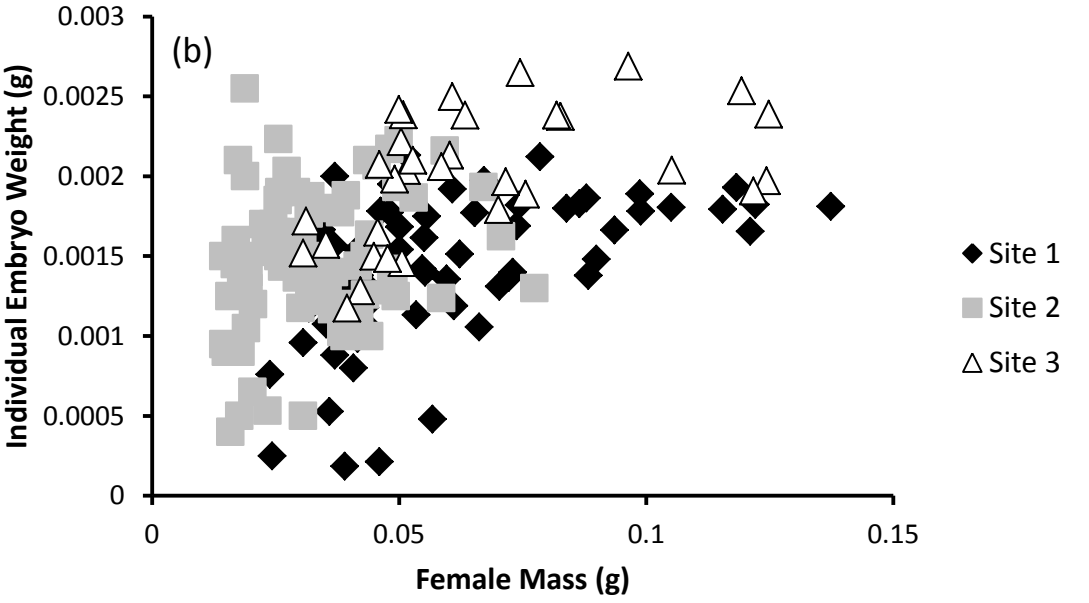
(b)

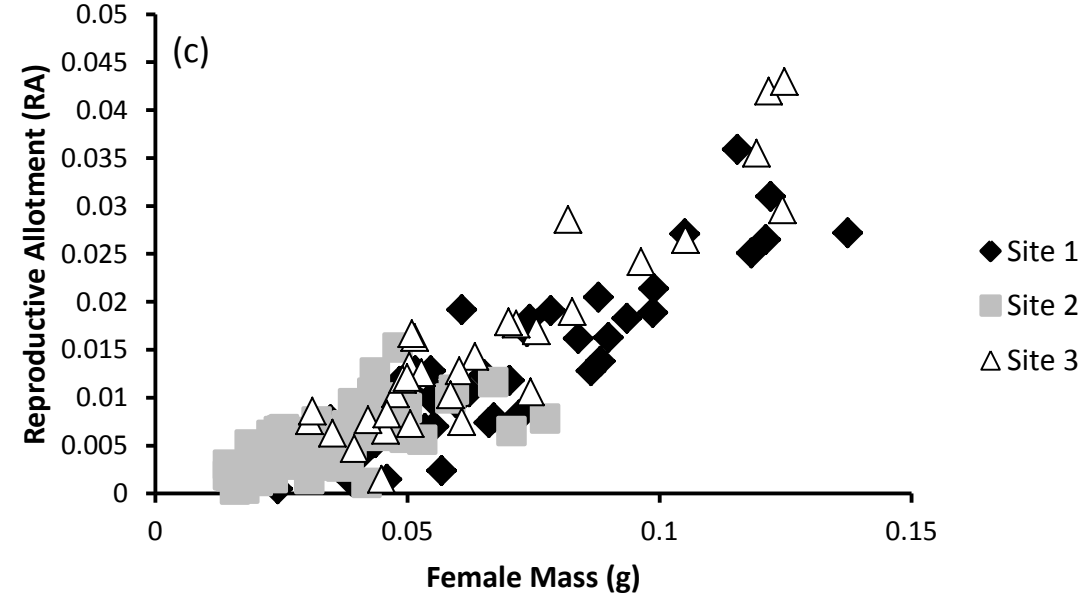

2
36
100%
90%
80%
70%
60%
50%
40%
30%
20%
10%
0%
Percentage of females
January 2012
April 2012
Month
Non-reproductive
Reproductive

(c)


(a)
Total Number of Embryos (Brood Size)
7.0
6.5
6.0
5.5
5.0
4.5
4.0
3.5
3.0
1
2
3
Site
(b)
Individual Weight of Embryos (g)
0.0024
0.0022
0.0020
0.0018
0.0016
0.0014
0.0012
0.0010
0.0008
1
2
3
Site
(c)
Reproductive Allotment (g)
0.013
0.012
0.011
0.010
0.009
0.008
0.007
0.006
0.005
0.004
0.003
1
2
3
Site
(d)
Female Size at Maturity (mm SL)
30
28
26
24
22
20
18
16
14
12
10
1
2
3
Site
(e)
Male Size at Maturity (mm SL)
30
28
26
24
22
20
18
16
14
12
10
1
2
3
Site
(f)
Matrotrophy Index (MI)
18
16
14
12
10
8
6
4
2
0
1
2
3
Site
January 2012
April 2012
March 2013


(a)
Total Number of Embryos (Brood Size)
25
20
15
10
5
0
0
0.05
0.1
0.15
Female Mass (g)
Site 1
Site 2
Site 3


(b)
Individual Embryo Weight (g)
0.003
0.0025
0.002
0.0015
0.001
0.0005
0
0
0.05
0.1
0.15
Female Mass (g)
Site 1
Site 2
Site 3


(c)
Reproductive Allotment (RA)
0.05
0.045
0.04
0.035
0.03
0.025
0.02
0.015
0.01
0.005
0
0
0.05
0.1
0.15
Female Mass (g)
Site 1
Site 2
Site 3