Natural history and life history of the Grijalva gambusia *Heterophallus milleri*
Radda, 1987 (Teleostei: Poeciliidae)

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Abstract
We report on basic natural history and life history data of the Grijalva gambusia *Heterophallus milleri* (Poeciliidae) from a small creek in Tabasco, southern México. Basic biology of *H. milleri* is similar to that of other members of the tribe Gambusini. Insects make up at least part of *H. milleri*’s diet, the tertiary sex ratio was female-skewed, female *H. milleri* produced small offspring, one clutch at a time, relied predominantly on yolk for embryo provisioning (MI: 0.73), and male size was normally distributed.

Zusammenfassung
Wir berichten über die Grundzüge der Naturgeschichte und Lebensgeschichte von Grijalva-Moskitofischen *Heterophallus milleri* (Poeciliidae) aus einem kleinen Creek in Tabasco, Südäquador. Grundsätzlich ähnelt die Biologie von *H. milleri* der anderer Arten des Tribus Gambusini. Insekten machen wenigstens einen Teil der Nahrung aus, im tertiären Geschlechterverhältnis überwogen die Weibchen, die weiblichen *H. milleri* brachten kleine Nachkommen hervor, jeweils ein Gelege, wobei die Embryos hauptsächlich von Dotter ernährt werden (MI: 0.73), die Größe der Männer lag im Bereich der Normalverteilung.

Résumé
Nous rendons ici compte des données essentielles de l’histoire naturelle et de la biologie de la gambusie *Heterophallus milleri* (Poeciliidae) originaire d’un petit ruisseau de Tabasco, au sud du Mexique. *H. milleri* possède une base biologique similaire à celle des autres membres de la tribu des Gambusini. Les insectes composent au minimum une partie du régime de l’espèce ; son sex-ratio tertiaire est à dominante femelle ; la femelle donne naissance, à chaque accouplement, à de petits alevins, reliés en grande partie au vitellus, à partir duquel l’embryon s’alimente (MI: 0.73) ; la taille des mâles était normalement répartie.

Sommario
Si riportano dati sulla storia naturale e sul ciclo vitale della gambusia di Grijalva *Heterophallus milleri* (Poeciliidae) di un piccolo torrente nello stato di Tabasco, Messico meridionale. La biologia di *H. milleri* è simile a quella di altri membri della tribù Gambusini. Gli insetti rappresentano almeno una parte della dieta di *H. milleri*, il rapporto tra i sessi terziario è spostato verso la femmina, questa da origine ad una prolifera ridotta, una nidiata alla volta, che si affida in modo predominante al tuorlo per l’avvistamento del l’embrione (MI: 0.73) e la taglia dei maschi ha una distribuzione normale.

INTRODUCTION
Life-history evolution has been an integral part of evolutionary research over the past few decades and livebearing fishes (Poeciliidae) have proven to be excellent models for studying life history adaptations (e.g., Reznick & Miles 1989; Magurran 2005). Originally, more than 200 species and 22-29 genera of poeciliids were endemic to the Americas, but due to human introductions they are now found worldwide (Lucinda 2003). Within the Poeciliidae, at least three characters evolved to give rise to the present diversity within the family: (1) internal fertilization using a transformed anal fin, the gonopodium, for sperm transfer, (2) livebearing, and (3) different degrees of maternal provisioning for the developing young (Constantz 1989; Reznick & Miles 1989). With the sole exception of *Tomeutes gracilis* Eigenmann, which is characterized by oviporous egg retention, internal fertilization and viviparity are found in all species of poeciliids.
(Rosen 1964). Furthermore, some poeciliids are able to accommodate several clutches simultaneously, so-called superfetation (e.g., Turner 1937; Thibault & Schultz 1978; Reznick & Miles 1989).

The Grijalva gambia, *Heterophallus milleri* Radda, 1987, is a small livebearing fish inhabiting the waters of the Río Grijalva basin in Tabasco, México (Miller 2005; Fig. 1). The genus *Heterophallus* is considered a sister genus to *Gambasia* within the tribe Gambusiini (Harbek et al. 2007), and comprises three different species (Radda 1987; Miller 2005). Nonetheless, besides the species description (Radda 1987) and two behavioral studies (Ziege et al. 2008; Riesch et al. 2009), the natural history of *H. milleri* and its sister species remains largely unknown. However, to fully understand the evolution of reproductive strategies in livebearing fishes (e.g., Reznick & Miles 1989; Pires et al. in press) and other taxa, it is of utmost importance to also collect data on smaller and lesser-known clades.

Here, we report on basic natural history and life history data that we collected on *H. milleri* over the last five years, while conducting fieldwork in Tabasco, southern México. Furthermore, we will report on some basic behavioral observations of *H. milleri* in their natural habitat and will describe the species communities of which they are a part.

**MATERIAL AND METHODS**

We conducted life-history dissections on a total of 75 individuals (23 males and 52 females) that were collected in the Arroyo Bonita (17°25’37.42”N, 92°45’6.98”W; Fig. 1), a small creek, and tributary to the Río Oxolotán near the village of Tapijulapa in Tabasco. Fish were collected using a combination of seining and dip netting and were preserved in 10% formaldehyde.

Following the protocol of Reznick & Endler (1982), all preserved fish were weighed and measured for standard length. Males were classified as mature based on the morphology of their modified anal fin (i.e. gonopodium). The anal fin in poeciliids undergoes a complex metamorphosis as fish attain maturity, and the endpoint of this metamorphosis provides a reliable index of sexual maturity, allowing us to determine the state of complete maturation based on external cues alone (e.g. Constantz 1989; Langerhans in press). We therefore consulted the illustrations of the fully developed gonopodium of *H. milleri* presented by Radda (1987).

The reproductive tissue (i.e., testes for males and ovaries for females) and, if present, all developing offspring were removed. Offspring were counted and their stage of development determined (Haynes 1995; Reznick et al. 2007). Somatic tissues, reproductive tissues, and embryos were then dried for 10 days at 40°C and weighed again. To assess male, female, and embryo condition, somatic tissues and embryos were rinsed six times for at least six hours in petroleum ether to extract soluble nonstructural fats (Heulett et al. 1995; Riesch et al. 2010a, b, 2011) and were then dried again and reweighed.

We thus measured standard length [mm], dry weight [g], lean weight [g], and fat content [%] for males and females, gonadosomatic index [GSI, %] for males only, and fecundity [number of developing offspring], reproductive allocation [RA, %], offspring dry weight [mg], offspring lean weight [mg], and offspring fat content [%] for females only. GSI is calculated as testis dry weight [g] divided by the sum of testis dry weight [g] and somatic dry weight [g]. RA, on the other hand, is calculated as the total dry weight of all developing young [g] divided by the sum of the total dry weight of all developing young [g] and somatic dry weight [g].

Finally, we calculated the matrotrophy index [MI] as a measure to indirectly evaluate post-fertilization maternal provisioning (e.g., Reznick et al. 2007; Riesch et al. 2010c). The MI equals the estimated dry mass of the embryo at birth divided by the estimated dry mass of the oocyte at fertilization. Thus, if the eggs were fully provisioned by yolk prior to fertilization (lecithotrophy), we would expect the embryos to lose 30-40% of their dry mass during development (MI ≤ 0.75; Pollux et al. 2009). On the other hand, in the case of continuous maternal provisioning even after fertilization (matrotrophy), one would expect the embryos to lose less mass (MI between 0.75 and 1.00) or even to gain mass during development (MI ≥ 1.00; Pollux et al. 2009).

**RESULTS**

Over the last five years, we caught *H. milleri* in a variety of habitats in Tabasco, ranging from small creeks [Arroyo Bonita and Arroyo Tacubaya (17°27’12.78”N, 92°47’4.16”W) near the village of Tapijulapa] to large rivers [Río Oxolotán (17°26’39.98”N, 92°45’46.55”W), Río Amatan (17°27’33.26”N, 92°46’42.53”W), and Río Teapa (17°33’24.90”N, 92°57’3.54”W)] (Fig. 1). Teleost fish species communities at these habitats are quite complex (Tobler et al. 2006; Riesch et al. 2009) and at Arroyo Bonita *H. milleri* share the habitat with *Pecilia mexicana* Steindachner, *Priapella chamulae*
Rüdiger Riesch, Timothy J. Colton, Brandon L. Joachim and Ingo Schlupp

Table I. Mean±SE of male and female life history traits for *Heterophallus milleri* caught in Arroyo Bonita (Tabasco, México) on January 12th, 2010. Values in parentheses provide min-max. GSI: gonadosomatic index; RA: reproductive allocation; MI: matrotrophy index.

<table>
<thead>
<tr>
<th>Sex</th>
<th>Sample size</th>
<th>SL [mm]</th>
<th>Somatic dry weight [mg]</th>
<th>Fat content [%]</th>
<th>Fecundity</th>
<th>Estimated embryo dry mass at birth [mg]*</th>
<th>Embryo fat content [%]</th>
<th>GSI [%]</th>
<th>RA [%]</th>
<th>MI</th>
</tr>
</thead>
<tbody>
<tr>
<td>male</td>
<td>23</td>
<td>21.65±0.39 (18–25)</td>
<td>41.39±2.71 (22–64)</td>
<td>5.61±1.11 (0–19)</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>2.36±0.13 (0.7–3.8)</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>female</td>
<td>49</td>
<td>23.76±0.29 (20–29)</td>
<td>51.43±2.30 (27–97)</td>
<td>3.29±0.37 (0–11)</td>
<td>-</td>
<td>-</td>
<td>1.224</td>
<td>16.15±0.48 (7–24)</td>
<td>18.87±0.80 (1–31)</td>
<td>0.74</td>
</tr>
</tbody>
</table>

* estimated dry mass at birth is calculated using the slope and constant from the regression between embryonic dry mass and stage of development.

Schartl, Meyer & Wilde, *Xiphophorus hellerii* Heckel, *Heterandria himaculata* Heckel (all Poeciliidae), *Astyanax aeneus* Günther (Characidae), *Atherinella alvarezi* Díaz Pardo (Atherinopsidae), *‘Cichlasoma salvinii* Günther, *Thorichthys helleri* Steindachner, *Vieja bifasciata* Steindachner (all Cichlidae), and *Gobiomorus dormitor* Lacépède (Elotheridae) (see also Tobler et al. 2006). After strong rainfalls in September of 2008, we even sighted a *Strongylura hubbsi* Collette (Belonidae) at Arroyo Bonita. Among these fishes, *H. milleri* is the only one (with the exception of *S. hubbsi*) that consistently swims close to the water surface. In smaller creeks like Arroyo Bonita *H. milleri* usually crisscrosses the full width of the water body, while it tends to stay within the calmer littoral zone in larger rivers (e.g., Río Oxolotán, or Río Teapa).

Although no actual gut content analysis was conducted, several individuals had insect remains in their gut (Fig. 2C) that were easily identifiable dur-

![Map of Mexico showing Arroyo Bonita and other locations](image)

**Fig. 1.** Overview of the general study area in México with reference cities in grey.
ing life history dissections. It therefore seems that at least part of _H. milleri_’s diet is made up of insects.

General life history data are compiled in Table I. _Heterophallus milleri_ at Arroyo Bonita was characterized by a sexual size dimorphism with males being smaller than females (Table I). The tertiary sex ratio was female-biased and approximately 1:2.3 (males: females; Table I). Almost all dissected females (94.2%) were reproductively active and embryo weight decreased with stage ($R^2 = 0.209$, $P < 0.001$) in a fashion corresponding to a predominantly lecithotrophic provisioning strategy (MI = 0.74; Fig.

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**Fig. 2.** _Heterophallus milleri_ Radda. (A) Male (top) and female (bottom); (B) ready-to-be-born embryos; (C) a beetle found in the gut of a _H. milleri_ specimen. A and B were stacked using CombincZM (by Alan Hadley).
Fig. 3. Scatter plot of mean embryo dry mass vs. stage of development. Stages are determined on a progressive scale with the earliest (5) being the neurula stage and the oldest (50) being equivalent to embryos that are ready-to-be-born (after Haynes 1995; Reznick et al. 2007).

Fig. 4. Size distribution of mature males [as defined by the morphology of the gonopodium (after Radda 1987)] for H. mili-leri. For visual clarification, a potential normal distribution is superimposed.
3). We did not find any evidence for superfetation in *H. milleri*, as all developing embryos of the same clutch were always of approximately the same developmental stage. Male size distribution in *H. milleri* from Arroyo Bonita did not deviate from a normal distribution (Kolmogorov–Smirnov test: \( D_{23} = 0.14, P = 0.200 \); Fig. 4).

**DISCUSSION**

Our own observations confirm previous accounts that described *H. milleri* to prefer the calmer nearshore regions of streams and creeks (Radda 1987; Miller 2005). *Heterophallus milleri* is clearly one of the smaller poeciliids, being similar in size to, e.g., *Cnesterodon iguape* Lucinda, *Gambusia eurystoma* Miller, *Gambusia sexradiata* Hubbs, *Phalloptychus januarius* Hensel, and *Microptelia picta* Regan (Reznick & Miles 1989; Riesch et al. 2010a; Pires et al. in press; R. Riesch, unpubl. data; Fig. 2A). Like many other life history traits, offspring size and fecundity are usually related to female body size (Reznick & Miles 1989). Hence, it is not surprising that *H. milleri* produce only relatively few and small offspring per clutch (Table I; Fig. 2B). Overall, life histories of *H. milleri* are similar to those of other members of the tribe Gambusiini (e.g., no superfetation; but see Pires et al. in press for citations on rare occurrences of superfetation in Gambusiini). In fact, offspring size at birth in Grijalva gambusia is comparable to some populations of the closely related *Gambusia affinis* Baird & Girard (e.g., Reznick & Miles 1989; Marsh-Matthews et al. 2005; R. Riesch, unpubl. data), and *G. sexradiata* (Riesch et al. 2010a). Likewise, offspring fat content matches that found in other Gambusiini from the same drainage (i.e., *G. sexradiata* and *G. eurystoma*,

*Heterophallus milleri*, male (top), female (above). Photos by D. Bierbach

*aqua* vol. 17 no. 2 - 26 April 2011 100
Rudiger Riesch, Timothy J. Colton, Brandon L. Joachim and Ingo Schlupp

Riesch et al. 2010a). Fecundity, on the other hand, is lower than in most G. affinis populations, but is similar to that in Brachyraphis episcopi Steinbachner, Gambusia marsha Minckley & Craddock, and Gambusia nuani Hubbs (Reznick & Miles 1989). Finally, RA appears to be slightly higher than in most Gambusini with the exception of G. affinis (Reznick & Miles 1989; Pires et al. in press) and G. sexradiata from the same drainage (Riesch et al. 2010a).

Males of many poeciliid species are characterized by a bimodal size distribution in natural populations (e.g., Kallman 1989; Kolluru & Reznick 1996; Reznick et al. 2007). Contrary to this, male size in Gambusini is usually normally distributed (e.g., Krumholz 1948), and congruently, this is what we found in H. milleri. Although this could be an indication that size at maturity in H. milleri is not genetically determined in a similar way as in certain other poeciliids (i.e., via the P-locus in swordtails; Kallman 1989), it is too early to clearly determine this, since interpopulation variation in male size distributions is common (e.g. Reznick et al. 2007), and environmental effects are known to sometimes overide genetic determination (e.g., Kolluru & Reznick 1996).

Finally, the sexual size dimorphism we report on here is typical for most poeciliids (Pires et al. in press). Furthermore, most natural populations of poeciliids are characterized by a female-skewed sex ratio (Snelson 1989) and H. milleri from Arroyo Bonita were no exception. Since secondary sex ratios are typically 1:1 in poeciliids, higher male mortality is usually thought to be the reason for a female-biased sex ratio at maturity (Snelson 1989).

The present study represents the first life history characterization of a poeciliid fish from the little known genus Heterophallus. While it already provides us with relevant insights into their life histories, it is important to keep in mind that the life histories we report on here were derived from specimens collected from a single site and point in time. Previous studies have clearly shown that life histories vary considerably between habitats and seasons in livebearing fishes (e.g., Reznick & Endler 1982; Reznick & Miles 1989; Riesch et al. 2010a-c, 2011). However, Arroyo Bonita is characterized by both, a teleost community and water chemistry, which are representative of similar environments in this part of southern México (Tobler et al. 2006, 2008; Riesch et al. 2009). We are therefore confident that the life history data presented in the current study is representative of H. milleri life-history strategies in a typical small creek environment. Nonetheless, H. milleri from larger rivers, such as the Rios Amatan, Oxolotan, and Teapa, are expected to differ considerably in life histories from those reported here, and more extensive studies (i.e., comparisons of H. milleri from different sample sites, or of laboratory-reared specimen with field-caught fish) are clearly warranted to help characterize the full breadth of life-history strategies exhibited by this species.

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