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Life history

Aspects of the life history of the Tamesí molly, *Poecilia latipunctata*, from two populations in the Río Tamesí drainage in northeastern Mexico

Aspectos de la historia de vida del topote del Tamesí, Poecilia latipunctata, en dos poblaciones de la cuenca de río Tamesí del noreste de México

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Abstract

In the face of human-induced environmental change, basic biological data for species threatened by the impacts of human activities are sorely needed to devise and execute proper conservation strategies. Here we provide aspects of basic life-history data for 2 populations of the critically endangered Tamesí molly *Poecilia latipunctata* (Poeciliidae), which is native to the headwaters of the Río Tamesí in northeastern Mexico. Furthermore, we compare their life history to that of 2 syntopic species, the Atlantic molly (*Poecilia mexicana*) and the Amazon molly (*Poecilia formosa*). Life histories of *P. latipunctata* are largely similar to that of other mollies: male size was normally distributed in both populations, and females produced 1 clutch at a time, while relying predominantly on yolk for embryo provisioning. However, at our 2 sample sites, *P. latipunctata* males were smaller than *P. mexicana* males, and *P. latipunctata* females produced significantly larger offspring than either *P. formosa* or *P. mexicana*. Based on patterns of male and offspring size, we cautiously suggest that, besides anthropogenic disturbance, *P. latipunctata* might also be suffering from strong interspecific competition, and we call on more research into the basic biology of this species (including its competitive ability).

Keywords: Competition; Endangered species; *Poecilia formosa*; *Poecilia mexicana*; Poeciliidae

Resumen

Frente al cambio ambiental inducido por el hombre, se requieren datos biológicos básicos para las especies amenazadas por los impactos de las actividades humanas para diseñar y ejecutar estrategias adecuadas de conservación. Aquí reportamos datos básicos de algunos aspectos de la historia de vida de 2 poblaciones del topote del Tamesí *Poecilia latipunctata* (Poeciliidae), nativa de los manantiales del río Tamesí en el noreste de México y catalogada en peligro crítico. Además, comparamos su historia de vida con la de 2 especies sintópicas, el topote del Atlántico (*Poecilia mexicana*) y el topote del Amazonas (*Poecilia formosa*). Las historias de vida de *P. latipunctata* son, en gran parte, similares a las de otros topotes: el tamaño del macho se distribuyó normalmente en ambas poblaciones y las hembras produjeron huevos de forma discreta, predominantemente con vitelo para el aprovisionamiento de embriones. Sin embargo, en nuestros 2 sitios de muestreo, los machos de *P. latipunctata* fueron más pequeños que los machos de *P. mexicana* y las hembras de *P. latipunctata* produjeron crías significativamente más grandes que *P. formosa* o *P. mexicana*. Basándonos en los patrones de tamaño de los machos y de los descendientes, sugerimos que, además de la perturbación antropogénica, *P. latipunctata* también esté sufriendo una fuerte competencia interespecífica, por lo que recomendamos más investigaciones sobre la biología básica de esta especie (incluida su capacidad competitiva).

Palabras clave: Competencia; Especies amenazadas; *Poecilia formosa*; *Poecilia mexicana*; Poeciliidae

Introduction

It is well-established that human activities are having a negative, sometimes catastrophic, impact on biodiversity and ecosystem functioning (Barnosky et al., 2011; Pimm et al., 2014). Aquatic ecosystems, for example, provide important ecosystem services that are closely tied to human well-being (Constanza et al., 2014; Geist, 2011), but are also exposed to a variety of human-induced changes, including the impacts of invasive species (Gozlan et al., 2010), pollution (Conkle et al., 2018) or ocean acidification (Sunday et al., 2017). This is having a drastic impact on aquatic biodiversity (Janse et al., 2015). However, in order to devise and employ proper conservation and species management plans, we first need to have a good understanding of the basic biology of the focal species, which, unfortunately, is often lacking (Stroud & Thompson, 2019).

While there are many different biological traits that can be investigated to gain a better understanding of the basic biology of a species under threat of extinction, life histories are of particular relevance in this regard (Stearns, 1992). For example, they tell us how organisms invest their acquired resources into growth, maintenance, and reproduction, but the specific life-history strategies employed by a species can also help us understand how they might interact with other syntopic species (Stearns, 1992). This makes life-history traits ideal for advancing our species-specific biological knowledge, because they help provide essential demographic data relating to fertility and population growth. This type of data can, for example, be used to gauge a species' competitive ability (Bashey, 2008).

The Tamesí molly, *Poecilia latipunctata*, is a small livebearing fish of the family Poeciliidae, is critically endangered, and native to headwaters of the Río Tamesí in northeastern Mexico (Contreras-Balderas & Almada-Villela, 1996; Tobler & Schlupp, 2009). The species has a relatively small range, which makes *P. latipunctata* especially vulnerable to habitat changes (Miller et al., 2005). The most important threats to the survival of this species stem from invasive species as well as fragmentation and deterioration of suitable habitat as a result of local agricultural practices (Tobler & Schlupp, 2009). Biologically, this species is of special interest for at least 3 reasons. First, despite the fact that it looks like a short-fin molly (Fig. 1A-E), it actually belongs to the long-fin mollies (Ptacek & Breden, 1998; Scharl et al., 1995). Second, it is sympatric with 2 other mollies, the Atlantic molly (*Poecilia mexicana*) and the Amazon molly (*Poecilia formosa*), and has been reported to be one of the few natural sperm donors for *P. formosa* (Niemeitz et al., 2002), an all-female hybrid species that reproduces via gynogenesis (i.e., sperm-dependent parthenogenesis; Schlupp & Riesch, 2011). This role is critical as *P. latipunctata* is a sperm donor species that was not involved in the hybridization event that led to Amazon mollies, and the mating system in the area of Ciudad Mante is one of very few described to have more than 1 sperm donor species (Joachim & Schlupp, 2012; Schlupp, 2009). Third, it is also invasive in at least 1 habitat outside its natural range. While it seems that an introduction into Florida, USA, has failed (there are no confirmed observations since 1971; Neilson, 2019), it has established a thriving population in La Media Luna, San Luis Potosí, México (Miller et al., 2005; Palacio-Núñez et al., 2015).

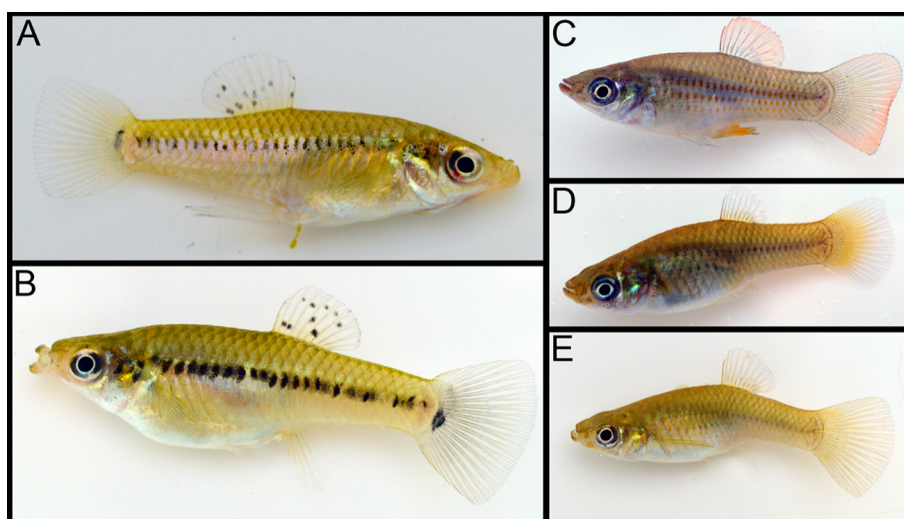


Figure 1. Male (A) and female (B) *Poecilia latipunctata*, male (C) and female (D) *Poecilia mexicana*, and female (E) *Poecilia formosa*. All fish laboratory-raised but originating from EL; pictures not to scale. Photos by Rodet Rodríguez Silva.

Nonetheless, very little is currently known about the basic biology of *P. latipunctata*, and how it might interact with syntopic *P. formosa* and *P. mexicana*. Here, we therefore report on basic life histories for 2 natural populations of *P. latipunctata*. Moreover, we compare aspects of male and female life histories of *P. latipunctata* to those of syntopic *P. mexicana* and *P. formosa*. With regards to the latter, we address 2 different questions: 1) What are some of the life-history differences between the 3 species? 2) Are these life-history differences similar or different between the 2 different population?

Material and methods

We collected fishes from 2 different localities in northern México, near Ciudad Mante in May 2010, and preserved all specimens in 10% formaldehyde under the guidelines granted in the collection permit from the Mexican government (SEMARNAT=SGPA/DGVS/02221/10). Due to the endangered species status of *P. latipunctata* we were only able to collect a small number of fishes. Furthermore, *P. latipunctata* has a small geographic range with low abundance and thus, the number of sites we could use for comparison was limited in our study (García-De León et al., 2018; Tobler & Schlupp, 2009). We therefore focused on 2 sites: one was located in the canalized headwaters of the Río Mante (Mante Canal or MC hereonafter: 22°42'54.79" N, 99°1'18.83" W; Río Guayalejo/Pánuco drainage). This is a wide channel that takes water a short distance from the spring to provide water for agriculture. The second was a small ditch in between sugarcane fields

within the same river drainage north of Ciudad Mante called El Limón (EL: 22°48'44.57" N, 99°0'44.55" W; Río Guayalejo/Pánuco drainage). At MC, we collected *P. latipunctata* and *P. mexicana*, while at EL, we collected all 3 species: *P. latipunctata*, *P. mexicana*, and *P. formosa*. Using a Hydrolab© Multisonde, we also measured several physicochemical characteristics: water temperature, pH, turbidity, oxygen content, salinity, chlorophyll and specific conductance. Our qualitative measurements included: the presence of a current, agriculture, predators, the type of substrate, contact vegetation, if the water level was managed, and the level of abundance of water plants (Table 1). All preserved specimens were then transferred to the University of Oklahoma in Norman, Oklahoma (USA) for further analyses.

To quantify selected life-history traits, fishes were dissected following well-established protocols (Riesch et al., 2015, 2016). In short, we first measured fish standard length (SL), and then removed testes for males and ovaries with (if present) developing embryos for females. Fishes and dissected reproductive tissues were then dried for 24 hrs at 55 °C, after which they were weighed to establish dry weights. Soluble fats were extracted with a petroleum ether bath, then we weighed all fish and tissues again to establish lean weights. Thus, we quantified the following traits: male and female SL (mm), male and female dry and lean weights (g), male and female fat content (%; relative to dry weight), fecundity (number of fertilized and developing oocytes), offspring dry and lean weight (mg), and offspring fat content (%; relative to embryo dry weight). We further calculated the gonadosomatic index (GSI) for males [testis dry weight / (testis dry weight +

Table 1

Descriptive statistics for single-point biotic and abiotic parameters collected at El Limón and Mante Canal at the same time as fish were caught for this study. The following variables were measured: quantitatively = water temperature (°C), specific conductivity (SpC), pH, dissolved oxygen (DO), chlorophyll *a* (Chloro), and salinity; qualitatively = the presence of a current; the presence of agriculture, the types of fish predators, the substrate type, the type of contact vegetation, if the water level is managed, and the abundance level of water plants.

Biotic/abiotic variable	Field sites	
	El Limón	Mante Canal
Water temperature (°C)	28.45	27.26
SpC (mS/cm)	1.479	1.008
pH	7.84	7.48
DO (% Saturation)	35.65	59.55
DO (mg/l)	2.60	4.77
Chloro (ug/l)	1.28	3.30
Salinity (0/00)	0.79	0.53
Current present	Yes	Yes
Agriculture present	Yes	Yes
Fish predators	Mexican tetra	Cichlids
Substrate type	Sand	Sand, silt
Contact vegetation	Herbs, shrubs, trees	Herbs
Water level managed	Yes	Yes
Water plant abundance	Low	High

somatic dry weight)] and reproductive allocation (RA) for females [total offspring dry weight / (total offspring dry weight + somatic dry weight)]. Finally, we evaluated the reproductive mode (i.e., if these poeciliids are capable of superfetation, which is the existence of more than a single brood at the same time; Pires et al., 2011).

For females, we further calculated the matrotrophy index [MI] to indirectly evaluate rates of post-fertilization maternal provisioning. The MI equals the estimated dry weight of the embryo at birth divided by the estimated dry weight of the oocyte at fertilization (Pollux et al., 2009). 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 weight during development ($MI \leq 0.75$). On the other hand, in the case of continuous maternal provisioning even after fertilization (matrotrophy), one would expect the embryos to lose less weight (MI between 0.75 and 1.00) or even to gain weight during development ($MI \geq 1.00$; Pollux et al., 2009). Due to a low sample size, we did not calculate MI for *P. mexicana* females from MC nor for *P. formosa* from EL.

In preparation for additional subsequent analyses, we then log-transformed (SL and all lean weights), square-root transformed (fecundity) and arcsine-transformed (all

fat contents, GSI and RA) all variables, and subsequently z-transformed them to meet model assumptions and to remove scaling effects.

For statistical analyses, we conducted 2 sex-specific univariate general linear models (ANOVA) on SL. For the male model, the factors were population (EL or MC) and species (*P. latipunctata* or *P. mexicana*). Since we did not have any life-history data for *P. mexicana* from MC, we did not include the species-by-population interaction effect. For the female model, we included the factors population (EL or MC), species (*P. latipunctata*, *P. mexicana*, and *P. formosa*), and the species-by-population interaction.

In a second step, we then ran 2 sex-specific multivariate general linear models (MANCOVA) on the remaining life histories. For the male model, we included the traits lean weight, fat content and GSI as dependent variables, species and population were again the factors, and SL now served as a covariate. We further included the interactions between SL and the 2 factors in the initial model but removed any non-significant interactions with $p > 0.1$ from the final model. For the female model, we included the traits lean weight, fat content, fecundity, offspring lean weight, offspring fat content, and RA as dependent variables, species and population were the factors, with SL and offspring stage of

development now served as covariates. We further included all possible two-way interactions between the factors and covariates in the initial model, but again removed any non-significant interactions ($p > 0.1$) from the final model.

All tests were conducted in IBM® SPSS® Version 21 (IBM Corp. 2012). Assumptions of normality of residuals and homocedasticity were met for all models.

Results

Descriptive statistics for life histories of males and females can be found in table 2. *Poecilia latipunctata* and *P. mexicana* were characterized by a pronounced sexual size dimorphism, with males being smaller than females.

Males. Male size distribution in *P. latipunctata* from EL and MC and in *P. mexicana* from EL did not deviate from normal distributions (Shapiro-Wilk test, *P. latipunctata*, EL: $W_{15} = 0.951$, $p = 0.536$; MC: $W_{14} = 0.892$, $p = 0.087$; *P. mexicana*, EL: $W_{10} = 0.968$, $p = 0.876$; Fig. 2A-C).). Our ANOVA on male SL uncovered a significant species effect, because *P. mexicana* males were larger than *P. latipunctata* males (Tables 2, 3). Our multivariate analysis of male lean weight, fat content and GSI revealed a significant effect of the covariate SL, but the species—and population—factors were also significant (Table 3). Post-

hoc ANCOVA revealed that SL had a significant effect on lean weight ($F_{1,35} = 1663.629$, $p < 0.001$) and GSI ($F_{1,35} = 16.441$, $p < 0.001$), which both increased with increasing SL. Significant species—and populations—factors were only uncovered for fat content ($F_{1,35} = 13.191$, $p = 0.001$ and $F_{1,35} = 20.028$, $p < 0.001$, respectively). At EL, *P. mexicana* had more body fat than *P. latipunctata*, and *P. latipunctata* from MC had more body fat than conspecifics from EL (Table 2). GSI further exhibited a non-significant trend between species ($F_{1,35} = 4.061$, $p = 0.052$), with *P. latipunctata* showing a trend for a lower investment into reproduction (estimated marginal means of GSI corrected for SL = 33.33 mm, *P. latipunctata*, EL: 0.83%; MC: 0.91%; *P. mexicana*, EL: 1.10%).

Females. The ANOVA on female SL only found a significant effect of the interaction species-by-population, because *P. latipunctata* females were smaller than *P. mexicana* females in EL, but this pattern was reversed in MC (Table 4). In the MANCOVA on the remaining female life-history traits, we uncovered significant effects of the covariate SL as well as the factors species and population, while embryonic stage of development was not significant (Table 4). Post-hoc ANCOVA revealed that SL had a significant effect on female lean weight, fecundity, and embryo lean weight (all 3 traits increased with increasing

Table 2

Descriptive statistics (mean \pm SD) for life-history traits of male and female *P. formosa*, *P. latipunctata*, and *P. mexicana* from El Limón (EL), and Mante Canal (MC) in northeastern Mexico. GSI: gonadosomatic index; RA: reproductive allocation.

Species	Population	Sex	N	SL [mm]	Lean weight [g]	Fat content [%]	Fecundity	Embryo lean weight [mg]	Embryo fat content [%]	GSI/RA [%]
<i>P. formosa</i>	EL	♀	5	46.40 \pm 8.65	0.500 \pm 0.255	2.26 \pm 2.03	22.20 \pm 14.31	3.20 \pm 0.42	17.47 \pm 2.61	14.37 \pm 4.63
<i>P. latipunctata</i>	EL	♂	15	33.80 \pm 5.56	0.187 \pm 0.094	0.90 \pm 1.34	-	-	-	0.85 \pm 0.27
		♀	11	38.09 \pm 3.42	0.280 \pm 0.087	1.37 \pm 1.43	9.18 \pm 2.27	5.00 \pm 1.04	17.24 \pm 2.00	16.51 \pm 2.80
	MC	♂	14	30.29 \pm 5.93	0.145 \pm 0.112	5.30 \pm 3.23	-	-	-	1.10 \pm 0.49
		♀	9	45.33 \pm 2.45	0.445 \pm 0.088	6.90 \pm 6.26	21.89 \pm 10.99	4.81 \pm 0.93	17.42 \pm 2.58	19.64 \pm 6.76
<i>P. mexicana</i>	EL	♂	10	39.30 \pm 5.62	0.299 \pm 0.132	5.37 \pm 4.62	-	-	-	0.92 \pm 0.25
		♀	15	45.07 \pm 6.88	0.444 \pm 0.213	3.23 \pm 3.58	23.93 \pm 15.73	3.10 \pm 0.56	16.16 \pm 5.87	15.18 \pm 5.29
	MC	♀	7	43.43 \pm 8.26	0.401 \pm 0.244	9.07 \pm 3.99	30.71 \pm 20.89	2.89 \pm 0.94	20.99 \pm 6.42	19.20 \pm 5.60

Table 3

Results of univariate analysis of variance (ANOVA) and multivariate analyses of covariance (MANCOVA) examining life-history differences between males of *Poecilia latipunctata* (from 2 populations: EL and MC) and *P. mexicana* (EL only). *F*-ratios in the MANCOVAs were approximated using Wilks' values, partial variance was estimated using Wilks' partial η^2 , interaction terms in brackets were removed ($p > 0.1$) from the final model.

Effect	F	df	<i>p</i>	Partial variance
(a) SL (ANOVA)				
Species	5.015	1, 36	0.031	
Population	3.273	1, 36	0.079	
(b) Male life histories (MANCOVA)				
SL	555.316	3, 33	< 0.001	0.981
Species	4.872	3, 33	0.006	0.307
Population	6.466	3, 33	0.001	0.370
(SL × species)	0.327	3, 31	0.806	
(SL × population)	1.700	3, 32	0.187	

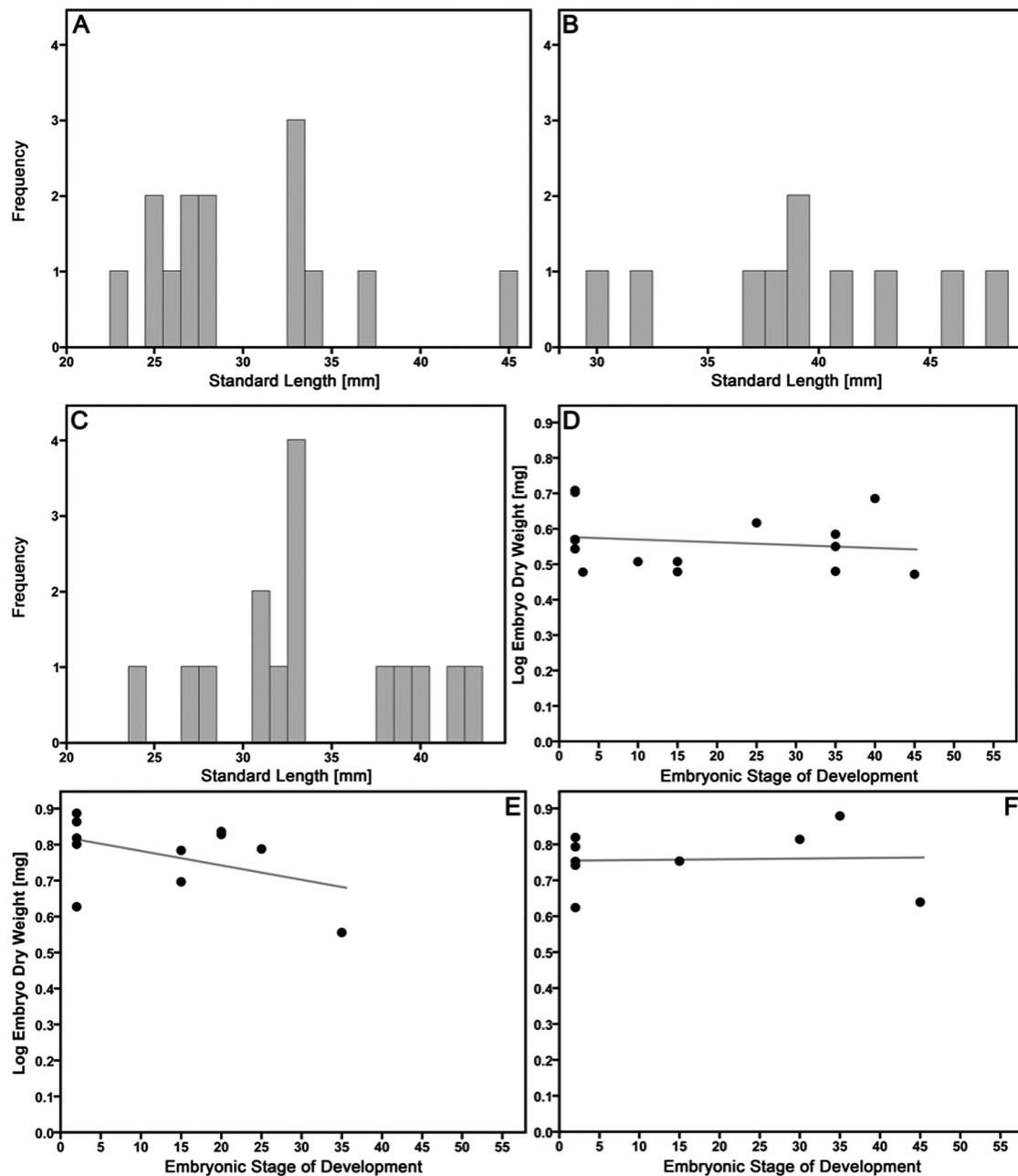
Table 4

Results of univariate analysis of variance (ANOVA) and multivariate analyses of covariance (MANCOVA) examining life-history differences between pregnant females of *Poecilia latipunctata* (from 2 populations: EL and MC), *P. formosa* (EL only) and *P. mexicana* (EL and MC). *F*-ratios in the MANCOVAs were approximated using Wilks' values, partial variance was estimated using Wilks' partial η^2 , and interaction terms in brackets were removed ($P > 0.1$) from the final model.

Effect	F	df	<i>p</i>	Partial variance
(a) SL (ANOVA)				
Species	1.909	2, 42	0.161	0.083
Population	2.371	1, 42	0.131	0.053
Species × population	6.242	1, 42	0.016	0.129
(b) Female life histories (MANCOVA)				
SL	170.052	6, 36	< 0.001	0.966
Stage of development	1.288	6, 36	0.287	0.177
Species	4.982	12, 72	< 0.001	0.454
Population	4.051	6, 36	0.003	0.403
(SL × species)	1.473	12, 68	0.156	
(Stage × species)	1.632	12, 64	0.106	
(SL × population)	1.783	6, 31	0.135	
(Species × population)	1.539	6, 30	0.200	
(Stage × population)	0.524	6, 29	0.785	

SL). Species differed significantly only in embryo lean weight ($F_{2,41} = 32.350$, $p < 0.001$) with *P. latipunctata* producing the largest offspring (EMMs of embryo lean weight at SL = 42.91 mm, *P. formosa*: 3.11 mg, *P. latipunctata*: 4.86 mg, *P. mexicana*: 2.88 mg), while lean

weight and fecundity only exhibited non-significant trends ($F_{2,41} = 2.896$, $p = 0.067$ and $F_{2,41} = 3.054$, $p = 0.058$, respectively). Populations differed significantly only in fat content ($F_{1,41} = 13.621$, $p = 0.001$), but there were also non-significant trends for fecundity ($F_{1,41} = 3.781$, p



Male standard length (mm) for: (A) *Poecilia latipunctata* from EL; (B) *P. latipunctata* from MC, and (C) *P. mexicana* from EL. (D-F) Scatterplots of mean embryo dry weight vs. stage of development (according to Riesch et al. 2011) for (D) *P. mexicana* from EL; (E) *P. latipunctata* from EL, and (F) *P. latipunctata* from MC.

= 0.059), embryo fat ($F_{1,41} = 3.188$, $p = 0.082$), and RA ($F_{1,41} = 3.071$, $p = 0.087$; Table 2).

Embryo dry weight either remained more or less constant or decreased strongly with developmental stage, which is congruent with a predominantly lecithotrophic provisioning strategy (MI, *P. latipunctata*, EL = 0.73; MC = 1.01; *P. mexicana*, EL = 0.90; Fig. 2D-F). This

confirmed the pattern we found for embryo lean weight (see above), because based on this, *P. latipunctata* produced larger neonates than *P. mexicana* (estimated dry weight at birth, *P. latipunctata*, EL = 4.00 mg, MC = 5.82 mg; *P. mexicana*, EL = 3.33 mg). We did not find any evidence for superfetation in *P. latipunctata*, *P. mexicana*, and *P. formosa*, as all developing embryos

per female were always of approximately the same developmental stage.

Discussion

As is typical for most poeciliids, *P. latipunctata* was characterized by strong sexual size dimorphism, with males being smaller than females (Pires et al., 2011). Moreover, size distributions for *P. latipunctata* did not deviate significantly from a normal distribution. At present it is too early to conclusively determine whether male size in *P. latipunctata* is genetically determined or not. However, bimodal size distributions in this family are usually strong evidence for size at maturity being genetically determined (Kallman, 1989), so this would suggest that size at maturity in *P. latipunctata* is not genetically determined. On the other hand, while males of some poeciliids exhibit a bimodal size distribution, the interpopulation variation in male size distributions is common, and environmental effects are known to sometimes supersede genetic determination (Kallman, 1989; Kolluru & Reznick, 1996; Reznick et al., 2007).

Overall, adult male and female life histories, as well as most offspring life histories were quite similar to those of other long-fin (e.g., *P. latipinna*: Martin et al., 2009; Riesch et al., 2012) and short-fin (e.g., *P. mexicana*: Riesch et al., 2010, 2011; this study) mollies (including the lack of superfetation; Pires et al., 2011). However, *P. latipunctata* was notably different from most other non-superfeting poeciliids from regular (i.e., non-extreme) aquatic habitats, in that they produced very large offspring. This is not fitting the general pattern within poeciliids well, whereby smaller species usually produce smaller offspring and larger species larger offspring (Pires et al., 2011). In fact, the offspring sizes we uncovered here for both populations, are much more similar to those found in non-superfeting poeciliids inhabiting toxic sulfide springs or dark caves (Riesch et al., 2010, 2011, 2016).

With respect to maternal provisioning, *P. latipunctata* was characterized by a largely lecithotrophic provisioning strategy (i.e., resources needed for embryo development are almost exclusively stored in the yolk prior to fertilization; Marsh-Matthews, 2011; Pires et al., 2011). However, similar to some previous studies in other poeciliids (Trexler, 1985; reviewed by Pires et al., 2011), we found inter-population variation in the extent of maternal provisioning as quantified via the MI (we have to point out, though, that sample sizes for estimating MI were quite low and late-stage embryos were largely missing). Overall, this is also congruent with the fact that some of the other male and female life histories differed to varying degree between these 2 populations. This suggests

potential differences in resource availability between EL and MC but could also result from genetic differences or other selective forces that differ between these 2 populations (Johnson & Bagley, 2011). Based on the abiotic parameters we measured at the time of sampling (Table 1), some of these differences could, for example, be based on the observed differences between habitats in conductivity, oxygen content, and chlorophyll a (a proxy for productivity) content. However, we do not know if these single point measurements represent more permanent differences between habitats. Moreover, both habitats are exposed to different levels of human disturbance, which could also have a measurable impact here. EL is a small drainage ditch in the midst of surrounding agriculture, mainly sugarcane fields, while MC is a fairly large irrigation channel in the midst of agricultural fields. We have observed EL to change over time due to removal of silt from the ditch, which temporarily turned the ditch into a relatively fast flowing habitat (which is not ideal habitat for poeciliids; Meffe & Snelson, 1989), but siltation and regrowth of plants returned the ditch quickly to a state that seemed more suitable for mollies.

Our sampling scheme allowed us to directly compare aspects of life histories between 3 different species (with respect to females) of poeciliid fishes that are all syntopic in the same 2 habitats in northeastern Mexico. For males, we could unfortunately only compare *P. mexicana* with *P. latipunctata* from EL due to sampling constraints (nonetheless, it is important to note that all 3 species did occur in both habitats). Here, male *P. mexicana* were significantly larger and also had significantly more body fat, suggesting that *P. mexicana* males might, at least at EL, have a competitive advantage over *P. latipunctata* males.

For females, we were able to compare all 3 species for EL, and *P. mexicana* and *P. latipunctata* at MC. At EL, *P. formosa* and *P. mexicana* were of roughly similar size, and also had very similar fecundities, RA, and produced offspring of roughly similar size. Given that *P. formosa* is a hybrid species originating from hybridization between *P. mexicana* and *P. latipinna*, this similarity is not necessarily surprising (Schlupp & Riesch, 2011). However, it is interesting to note that *P. latipunctata* females at EL were considerably smaller, and produced significantly larger but fewer offspring, resulting in a somewhat similar investment into reproduction (RA). We largely found the same similarities and differences when comparing *P. mexicana* and *P. latipunctata* females at MC, but in this population, *P. latipunctata* females were actually larger than *P. mexicana* females. The significantly larger offspring of *P. latipunctata* compared to the syntopic *P. mexicana* and *P. formosa*, suggest strong selection on offspring's competitive ability, as larger offspring have

been demonstrated to have better competitive abilities in poeciliid fishes (Bashey, 2008). Future experiments should investigate directly the relative competitive ability of the offspring of these 3 different species in these habitats.

The present study represents the first characterization of important life-history traits of the critically endangered *P. latipunctata*. While it already provides us with relevant insights into their life histories and reveals some similarities and differences relative to life histories of other mollies, more research into their basic biology is needed. One interesting aspect of *P. latipunctata* life histories was the large offspring size. If, as we speculate above, there is indeed strong competition between the 3 species (given their relatively similar niches, this is not necessarily surprising), this could be an additional factor on top of anthropogenic disturbance that might help explain why the species seems to be doing poorly in its native range. Additional studies from the area where *P. latipunctata* is invasive (La Media Luna), would be important in this context. We therefore call on more research into this species; without this it will be extremely difficult to plan and implement meaningful conservation efforts to save this charismatic little fish from extinction within its native range.

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