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Letter to the editor Open Access

Reproductive seasonality of *Astyanax mexicanus* cavefish

DEAR EDITOR,

The characiform fish *Astyanax* is a well-studied model system for understanding evolutionary development and genomics in cave biology. Nonetheless, considerable gaps remain in our understanding of its field ecology. Here, we conducted a four-year field study and found that while cavefish spawned year-round, reproductive peaks occurred in January to February.

The blind Mexican tetra (*Astyanax mexicanus*) (Figure 1A) is a major contributor to our understanding of the genetic and developmental factors controlling troglomorphic features and is one the most influential model systems in evolutionary developmental biology (Jeffery, 2012). The cavefish have a conspecific surface-dwelling morph that lives in nearby surface streams, with both morphs remaining inter-fertile. Thus, this species is well-suited for experimental manipulations. Morphological and behavioral attributes such as additional taste buds, higher chemosensory capabilities, increased number of cranial neuromasts, resistance to starvation, changes in immune system, and more efficient posture when bottom feeding (Jeffery, 2020), suggest the cave morph is better adapted to survive in dark habitats.

Different caves were visited to determine whether the presence of cavefish fry was seasonal, including Pachón Cave (visited on 20 March 2016, 22 May 2016, 6 August 2016, 21 November 2016, 8 January 2017, 16 March 2017, 14 April 2017, 12 August 2017, and 5 January 2020), Sabinos Cave (visited on 10 March 2017 and 19 February 2022), and Tinaja and Curva caves (visited on 22 March 2016). A description of each cave can be found in Elliott (2018). In Pachón Cave, most fish fry were observed in a small lateral pool, as described by Espinasa et al. (2017). Total fry number was estimated *in situ* through direct observation by *ad libitum* scanning of the pool surface (distance of ≤0.5 m) for approximately one hour per day. Abundance estimates were performed by the same person (L.E.) to avoid inter-individual differences in observational proficiency.

Post-larval fry were observed in Pachón Cave in all months of the year (Figure 1B) except for August 2017, likely due to limited visibility as a result of increased water level, inter-pool flow, and turbidity. The observed fry were 1.3–2.0 cm in length and scaleless, with a larval-like jaw and body shape, incompletely regressed eyes, and immature and small-sized adipose fins (Figure 1A), corresponding in morphology to 1.5–2.5-month-old post-larvae (Espinasa et al., 2017; Hinaux et al., 2011; Simon et al., 2017). As 1.5–2.5-month-old fry were observed year-round, some level of spawning must occur throughout the entire year.

The region has a dry season (January to April) and a rainy season (June to September) (Figure 1B). We measured the water temperature inside Pachón Cave. The lowest water temperature was recorded in December (24.6 °C) and the highest was recorded in August (25.4 °C). Although post-larvae were present throughout the year, our results also suggested there may be a preferred reproductive season. Notably, the number of observed fry was lowest during the rainy season, but showed a peak in abundance in March 2016 and 2017 during the dry season (Figure 1B). Assuming an age of 1.5–2.5-months, spawning of the March cohort would have occurred in January to February, when the dry season commenced and the water temperature began to increase from its lowest point. Exploration of Sabinos, Tinaja, and Curva caves also showed higher numbers of post-larvae in the same dry period as Pachón Cave, suggesting similar reproductive seasonality across all *Astyanax* cave populations.

Based on direct observations of two independent spawning events in Sabinos Cave on 19 February 2022, we confirmed the occurrence of spawning at the beginning of the dry season. While studying a group of fish, a presumed male and female were observed performing quiver swimming (a rapid synchronized swimming side-by-side), followed by an upward swimming and wrapping phase, which ended with a sudden upward swimming burst from the presumed female (under laboratory conditions, this is when the eggs are released). This behavior corresponds well to the spawning activity observed under laboratory conditions (Simon et al., 2019).

Following Espinasa et al. (2017), the gut contents of fry were obtained from six specimens collected on 21 March 2016 (1.5, 1.7, 1.7, 1.8, 1.9, and 2 cm), three specimens collected on 5 August 2016 (1.0, 1.7, and 1.7 cm), four specimens collected on 21 November 2016 (1.3, 1.4, 1.8, and 1.8 cm), and four specimens collected on 8 January 2017 (1.3, 1.6, 1.7, and 1.7 cm). Results showed that cladoceran water fleas were the main food item for the post-larval fry, consistent with previous research (Espinasa et al., 2017). On average, the post-larval fry ingested fewer water fleas in the rainy season (August=0.3±0.6 SD, n=3; November=0.0, n=4) than in the dry season (March=13.8±11.0, n=6; January=20.7±36.2, n=4). Harpacticoid copepods were the second most abundant food item and showed a similar trend (Figure 1C). In August and November 2016, average number of food items in the gut was

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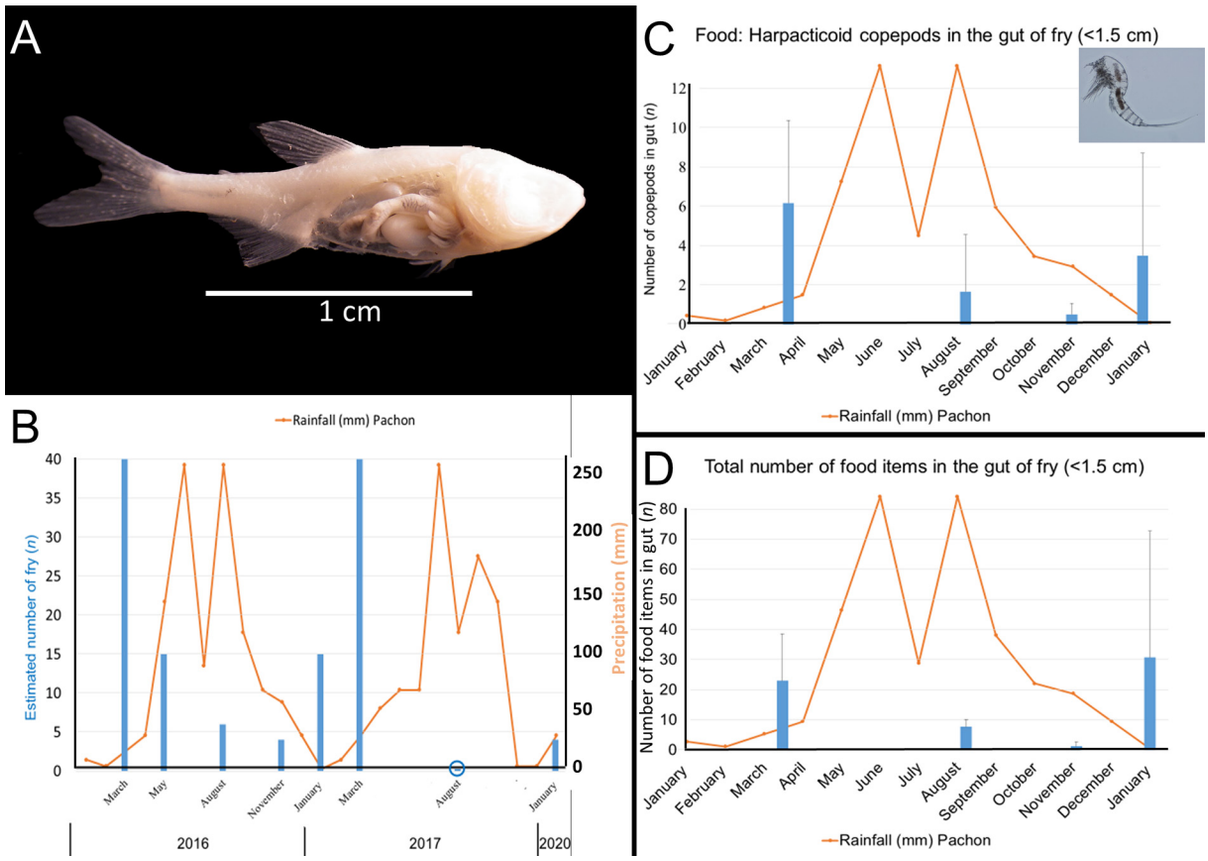

Figure 1 Reproductive seasonality of *Astyanax mexicanus* and prey abundance

A: Pachón Cave post-larval digestive system, showing size, body with no scales, larval-like jaw and body shape, incompletely regressed eyes, and adipose fins. This morphology corresponds to post-larval stages (approximately 1.5–2.5-months old). B–D: Monthly precipitation in Pachón Cave area was obtained from https://smn.conagua.gob.mx/es/ and plotted in orange. Precipitation scale in B is the same for C and D. B: Overlay of precipitation and number of fry observed in Pachón Cave (Blue columns). Post-larvae (approximately 1.5–2.5-months old) were found throughout the year at Pachón Cave, with a significant peak in abundance in March during the dry season. No error bars are included as data are single observation events. C: Average number of harpacticoid copepods in post-larval guts (Blue columns±*SD*). D: Average number of total food items in post-larval guts (Blue columns±*SD*). Food items in guts of cavefish fry are more abundant during the dry season. Peak spawning may be timed to coincide with highest microcrustacean food availability for fry.

7.7±2.5 and 1.2±1.5, respectively, while in March 2016 and January 2017, average number of food items was 23.0±15.5 and 30.7±41.9, respectively (Figure 1D). These findings suggest that although reproduction occurs year-round, peak spawning may be timed to coincide with higher microcrustacean food availability for the post-larvae. Alternatively, mortality may be higher during the rainy season due to lower food availability.

In the laboratory, cavefish spawning can be stimulated by raising the temperature. For example, Borowsky (2008) suggests that cavefish be maintained at 22 °C, with a gradual increase to 25 °C to promote spawning. At Pachón Cave, we found that the water temperature oscillated from 24.6 °C in January to 25.4 °C in August. In the laboratory, multiple spawning events can be achieved throughout the year by alternating temperatures. At Pachón Cave, water temperature can increase or decrease following local rain events. We propose that while most spawning may be triggered by warming temperatures and other environmental conditions associated with the onset of the dry season, local water temperature fluctuations may also stimulate reproduction throughout the year, resulting in the year-round presence of post-larval fry.

Previous studies of surface-dwelling *A. aeneus* (Trujillo-Jiménez et al., 2013) in the Champotón River (southeast Mexico) and *A. fasciatus* in the Furnas Reservoir (Brazil) (De Carvalho et al., 2009) reported that fish are reproductively active throughout the year, with a spawning peak at the time of highest temperature and rainfall. The prolonged reproductive period suggests that surface-dwelling *Astyanax* species are fractional spawners, mimicking our results for the cave-dwelling *A. mexicanus*.

SCIENTIFIC FIELD SURVEY PERMISSION INFORMATION

The collection permit (#SGPA/DGVS/02438/16) was obtained from SEMARNAT (México) by Patricia Ornelas García.

COMPETING INTERESTS

The authors declare that they have no competing interests.

AUTHORS' CONTRIBUTIONS

L.E. designed the study, collected and analyzed the data, and wrote the first draft of the manuscript. N.R. and S.R. participated in field trips. All authors obtained funding for the study and contributed substantially to revisions. All authors read and approved the final version of the manuscript.

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REFERENCES

Borowsky R. 2008. Breeding *Astyanax mexicanus* through natural spawning. *Cold Spring Harbor Protocols*,doi: 10.1101/pdb.prot5091.

De Carvalho PA, Paschoalini AL, Santos GB, et al. 2009. Reproductive biology of *Astyanax fasciatus* (Pisces: Characiformes) in a reservoir in southeastern Brazil. *Journal of Applied Ichthyology*, **25**(3): 306−313.

Elliott WR. 2018. The *Astyanax* Caves of Mexico: Cavefishes of Tamaulipas, San Luis Potosí, and Guerrero. Association for Mexican Cave Studies, 1–325.

Espinasa L, Bonaroti N, Wong J, et al. 2017. Contrasting feeding habits of post-larval and adult *Astyanax* cavefish. *Subterranean Biology*, **21**: 1−17.

Hinaux H, Pottin K, Chalhoub H, et al. 2011. A developmental staging table for *Astyanax mexicanus* surface fish and Pachón cavefish. *Zebrafish*, **8**(4): 155−165.

Jeffery WR. 2012. *Astyanax mexicanus*: a model organism for evolution and adaptation. *In*: White WB, Culver DC. Encyclopedia of Caves. 2nd ed. Waltham: Academic Press, 36–43.

Jeffery WR. 2020. *Astyanax* surface and cave fish morphs. *EvoDevo*, **11**: 14.

Simon V, Elleboode R, Mahé K, et al. 2017. Comparing growth in surface and cave morphs of the species *Astyanax mexicanus*: insights from scales. *EvoDevo*, **8**: 23.

Simon V, Hyacinthe C, Rétaux S. 2019. Breeding behavior in the blind Mexican cavefish and its river-dwelling conspecific. *PLoS One*, **14**(2): e0212591.

Trujillo-Jiménez P, Sedeño-Díaz JE, Camargo JA, et al. 2013. Reproductive traits and population structure of *Astyanax aeneus* (Characiformes: Characidae) from a subtropical river in Mexico. *Revista de Biología Tropical*, **61**(2): 769−786.