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Distinctive reproductive tactics between sympatric specimens of *Astyanax* aff. *fasciatus* in the wild and in captivity

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Abstract *Astyanax fasciatus* is a group of neotropical fish with two different diploid chromosome numbers, and in the wild, these individuals are found in sympatry. The annual reproductive cycles of adult females of species with different numbers of chromosomes (G1: 46 chromosomes, low responsiveness to induced spawning; G2: 48 chromosomes, responsive to induced spawning) were investigated in the wild and in captivity. Plasma estradiol (E2) levels, relative fecundity (RF), oocyte diameter, gonadosomatic index, and ovarian morpho-histology were evaluated to identify the ovarian maturation stage. The G1 females in both environments began the vitellogenic phase during winter, concurrent with increased plasma E2 levels. In wild G1 females, large numbers of vitellogenic oocytes were observed during spring and summer, when plasma E2 levels remained high. In contrast, captive G1 females showed higher E2 levels during the winter. They also retained significantly higher RF and vitellogenic oocyte numbers than wild females, but showed no spawning and slow yolk reabsorption. Wild G2 females began the vitellogenic phase in autumn and had progressively increasing E2 levels that peaked in the spring, with spawning occurring during the summer. In captive G2 females, the E2 levels and RF remained high throughout the year. These data suggest that *A.* aff. *fasciatus* with different numbers of chromosomes differ in their reproductive performance in the wild and show altered sensitivity to environmental cues in captivity. Our results indicate that G1 females likely respond best to artificial spawning just after winter, whereas G2 females should respond year-round.

Keywords Estradiol · Fecundity · Karyotype · Lambari · Reproduction

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Introduction

The construction of dams for hydropower generation and water supply affect the reproductive cycles of migratory fish species (Coleman et al. 2018). To reduce the aquatic environmental impacts caused by the dams, hydropower companies have established hatcheries to artificially spawn native fish species for use in restocking programs (Bai et al. 2017). This practice has been done with species of the genus *Astyanax* (Characidae), commonly called lambari, which is the largest neotropical characiform genus, containing approximately 259 described species (Eschmeyer 2013).

Astyanax fasciatus comprises a species complex that includes individuals with different diploid chromosome numbers (Morelli et al. 1983; Pazza et al. 2006, 2007, 2008; Artoni et al. 2008; Pansonato-Alves et al. 2013). The species in this group present reproductive plasticity based on environmental cues, displaying either asynchronous or synchronous oocyte development (Carvalho et al. 2009).

Two sympatric species of the *A. fasciatus* group occur in the headwaters of the Tietê River (São Paulo, Brazil), *Astyanax* cf. *fasciatus* and *Astyanax* sp. (Marceniuk et al. 2011). These two *Astyanax* have different diploid chromosome numbers (2n = 46 and 2n = 48), 18S rDNA distributions, *rex3* elements, constitutive heterochromatin patterns, and sequencing data for the cytochrome *c* oxidase subunit I gene (Pansonato-Alves et al. 2013). In addition, the sympatry, lack of hybridization, and different responses to induced reproduction in captivity suggest they are two different species yet to be taxonomically described. The literature lacks information about the responses of these species to artificial reproduction, but our previous experience has shown they respond differently to induced reproduction in captivity. *Astyanax* cf. *fasciatus* (2n = 48) responds satisfactorily to hormonal induction, while *Astyanax* sp. does not respond to the same spawning protocol (unpublished results).

According to Gerstein and Berman (2015), karyotype variations can occur in different populations of the same species, and these differences may involve not only the number of chromosomes but also the chromosomal structure. In the *Astyanax* genus, these sorts of ploidy variations can be found in *A. bimaculatus*, *A. scabripinnis*, and *A. fasciatus* (Morelli et al. 1983; Moreira-Filho and Bertollo 1991; Almeida-Toledo et al. 2002; Pazza et al. 2007, 2008; Artoni et al. 2008). Ploidy differences also occur in *Hoplias malabaricus* (trahira), a neotropical freshwater teleost with great potential for aquaculture (Faria et al. 2019) that presents seven general cytotypes clearly identifiable based on their diploid number (Bertollo et al. 2000). However, to our knowledge, no published data have shown a relationship between diploid chromosome number differences within the *Astyanax fasciatus* group and differences in reproductive physiology.

Therefore, the aim of this study was to evaluate the reproductive cycle of females from two cryptic *A. fasciatus* species in the wild and in captivity, testing the hypothesis that the emergence of the two diploid chromosome numbers may be associated with different reproductive tactics in this species.

Materials and methods

Experimental design

Ninety-six adult *A. fasciatus* females were collected over the course of a year. Six individuals of each chromosome cytotype (G1: 2n = 46; G2: 2n = 48) were sampled during each season (spring, summer, autumn, winter) from wild populations (23°34′36.5″ S; 45°54′23.9″ W). Likewise, captive fish (23°35′33.8″ S; 45°58′09.1″ W) from both cytotypes were sampled during the same periods, i.e., 6 captive specimens from each season and group (G1/G2) for a total of 48 animals. The captive fish were born in a fish farm and maintained in 100 m^2 ponds for two years under natural photoperiods and temperatures; they were fed twice daily with commercial extruded fish feed (Guabi®; 4 mm, 30% crude protein). The relative fecundity (RF), oocyte diameter, morphohistology of the ovaries, and plasma estradiol (E2) levels were analyzed throughout the year.

The sampled fish were anesthetized with 1 g benzocaine (previously diluted in 10 mL ethanol) in 10 L of water. Blood samples were collected by puncturing the caudal vein with disposable heparinized syringes and needles (Hepamax® S 5000i). The blood samples were centrifuged at 655.1 *g* for 5 min, and the plasma was frozen at −80 °C until processing. After blood collection, the length (cm) and body mass (g) were recorded. The females were subsequently euthanized by sectioning the spinal cord at the level of the operculum (Ethics Committee no. 172/2012, IB/USP). The ovaries were removed and weighed to calculate a gonadosomatic index [GSI = (gonad mass/body mass) × 100], and a portion of the ovaries was stored for histological analyses and calculating the relative fecundity and oocyte diameter.

Histological analyses

To confirm the gonadal maturation stage, samples from the middle third of the ovaries were fixed in Bouin's solution for 24 h and then transferred and held in 70% ethanol. For the histological procedure, samples were

dehydrated in increasing concentrations of ethanol, cleared in xylene (dimethylbenzene), and embedded in Paraplast® according to routine histological procedures. The samples were then sectioned using a microtome (Leica RM2255 equipped with disposable razors). Nine semi-serial sections (5 μm) for each specimen were placed on slides and stained with hematoxylin and eosin. After staining, the slides were dehydrated, diaphanized, and covered with a coverslip and Damar gum. The sections were imaged and analyzed using a Leica microscopy system (Leica DM1000 light microscope and Leica DFC295 camera; photo-documented using Leica Application Suite Professional, LASV3.6).

The maturation stage of the ovaries was classified according to the developmental stage of the follicles based on the cell types in each section. Based on Murua and Saborido-Rey (2010), the stages were established as being in the final-growth period, ripe, or spawned.

Determining oocyte diameter and relative fecundity

Relative fecundity is defined as the total number of vitellogenic and advanced yolked oocytes in the ovary, i.e., the oocytes that if ovulated could be fecundated (Murua et al. 2003). To analyze fecundity and oocyte diameter, pre-weighed ovary samples were fixed with Gilson solution, retained in the solution for 60 days to dissolve the ovarian tissue, and then stored in 70% ethanol. The same Leica microscopy system was used to measure and count the oocytes using 2 mL subsamples in triplicate with the aid of a Stempel pipette (Hensen Stempel Pipette™ 1806 series; Wildlife Supply Company, Florida, USA). The oocyte diameters were divided into 11 classes of 100 μm increments (0–100 μm, 101–200 μm, …, 1001–1100 μm), and the proportion of oocytes in each diameter class was determined. For oocytes with diameters >600 μm, information from the histological analyses and the oocyte density under a stereomicroscope were used for the vitellogenic classification. After measuring and counting the oocytes, relative fecundity (RF) was gauged in relation to body mass using the following formulas:

- Total number of oocytes per female = (number of oocytes counted in Gilson's solution × total gonad mass) / mass of ovaries placed in the Gilson's solution
- Absolute fecundity (AF) = (% vitellogenic oocytes × total number of oocytes per female) / 100
- Relative fecundity = AF / body mass
- Plasma estradiol levels
- Plasma E2 levels were measured using a commercial ELISA kit (IBL International, Hamburg, Germany). To validate the use of the kit, preliminary tests were conducted to establish the proper plasma dilutions. The analyses were then performed in duplicate according to the manufacturer guidelines using plasma dilutions of 1:2 or 1:4 depending on the experimental group and stage of the reproductive cycle. The intra-assay coefficient of variation (CV) calculated for sample duplicates in the same plate was 10.3%, while the inter-assay CV calculated for samples analyzed in different plates was 17.2%.

A parallelism test was also conducted with animals from the G1 and G2 groups. Plasma samples were serially diluted in buffer (1:2; 1:4; 1:8; 1:16, and 1:32) and assayed in the same plate with standard curve samples. The r^2 of the curve was calculated for the two groups (G1 and G2) and the standard curve, with an r^2 of >0.90 set as the pass limit for the linearity test. The r^2 values were 0.9999 ± 0.0001 for the standard curve, 0.9954 ± 0.0031 for G1, and 0.9905 ± 0.0111 for G2.

The detection limit of the test was 9.7 pg/mL (IBL International, Hamburg, Germany). Absorbance was measured using a microplate reader (Spectra Max 250 Molecular Devices) at 450 nm. A total of five plates were used to run the experimental samples (including the dilution tests and CV calculations), and another plate was used to run the parallelism test.

Statistical analysis

Data are expressed as the mean ± SEM (standard error of the mean). Body mass, GSI, RF, oocyte diameter, and E2 concentrations were compared with a general linear model analysis followed by Bonferroni post hoc tests with the factors "Environment" (captive and wild), "Season" (spring, summer, autumn, and winter) and "Group" (2n = 46 and 2n = 48) and their interactions. Fecundity was normalized to body mass. The E2 concentrations were $\log_{10}$-transformed to normalize the data, but they are presented in the original concentrations in the tables. In all analyses, the significance level was

95% ($P < 0.05$). The statistical analyses were performed using IBM SPSS Statistic 22.

Results

Biometric data

The mean body mass of the G1 animals throughout the year was 52.36 ± 13.16 g in captivity and 28.7 ± 3.7 g in the wild, while for the G2 animals, the body mass was 29.2 ± 7.2 g in captivity and 13.8 ± 2.1 g in the wild. In both groups and for all seasons, the animals in captivity were larger than those in the wild ($P < 0.001$). With the exception of the wild animals during the summer ($P = 0.095$), the G1 animals were larger than the G2 animals both in captivity and in the wild ($P < 0.001$).

The GSI in the wild females was higher for the G2 animals than for the G1 animals during the summer and autumn ($P < 0.001$ and $P < 0.002$, respectively). The GSI data also showed different temporal patterns between environments. Except for a significant difference between the spring and autumn ($P = 0.004$), the wild G1 females had a constant GSI value for most of the year. In contrast, captive G1 females had constant GSI values year round. Significant differences were observed between the two environments during summer and autumn, with higher GSI values for the animals in captivity ($P = 0.001$ for both; Table 1). There were no significant differences between the seasons within the same group and environment.

Wild G2 females showed an increase in their GSIs in the summer and a significant decrease in the winter ($P = 0.042$). In contrast, the captive G2 females maintained constant GSI values throughout the year. Significant differences were also observed between the two environments during the winter ($P = 0.008$), when the GSIs for the specimens in captivity were higher than those for the wild animals (Table 1).

Histological analyses

A descriptive analysis of the ovaries showed the presence of different follicular developmental stages, which allowed for the characterization of their maturation. The oocytes found during the microscopic examination were perinucleolar (PN; Fig. 1a), alveolar cortical (AC; Fig. 1b), vitellogenic (V; Fig. 1c), and atretic (A; Fig. 1d). Nuclear migration (Fig. 1e) was detected in the vitellogenic oocytes as well as a structure called a post-ovulatory follicle (POF; Fig. 1f). The stages were classified as follows:

- Final-growth period: Characterized by the large number of vitellogenic oocytes without nuclear migration. Perinucleolar, alveolar cortical, and atretic oocytes were also present. Many animals were found at this stage in the wild and in captivity throughout the year.
- Ripe: Also characterized by a large number of vitellogenic oocytes, but some showed nuclear migration. Perinucleolar, alveolar cortical, and atretic oocytes were also present. Wild G2 animals were found at this stage during the winter.
- Spawned: Only observed in the wild, this stage was characterized by some vitellogenic oocytes with nuclear migration (demonstrating a breakdown in the germinal vesicle) and POFs, indicating the ovulation of some oocytes; perinucleolar, alveolar cortical, and atretic oocytes was also present. This stage was found only in the wild females, in G1 animals during the autumn and in G2 animals during the spring and summer.

Wild G1 females had a significant number of perinucleolar oocytes during all periods analyzed and many vitellogenic oocytes in the spring (Fig. 2a), summer (Fig. 2c), and winter (Fig. 2g); these females were therefore classified as in the final-growth period. Vitellogenic oocytes were not present in the autumn, but post-ovulatory and atretic follicles were present (Fig. 2e); therefore, these fish were classified as spawned. In captivity, the females were in the final-growth period (without nuclear migration) of maturation throughout the year (Figs. 2b, d, f, and g), with vitellogenic oocytes present in all seasons.

The wild G2 females were classified as spawned during the spring (Fig. 3a) and summer (Fig. 3c), when they presented with POFs and nuclear migration. In the autumn, when these structures were absent, they were classified as in the final-growth period (Fig. 3e). During the winter, nuclear migration was observed but POFs were not found, so the ovaries were classified as in the ripe stage (Fig. 3g). The captive G2 group remained in the final-growth stage throughout the year, having significant numbers of vitellogenic oocytes without nuclear migration and no POFs present (Figs. 3b, d, f, and h).

Table 1 Gonadosomatic index (GSI), Estradiol concentration (E2) and Relative Fecundity (RF) values of *A. fasciatus* females of G1 (2n = 46) and G2 (2n = 48) collected in the wild and held in captivity throughout the year

	Wild				Captivity			
GSI (%)								
	Spring	Summer	Autumn	Winter	Spring	Summer	Autumn	Winter
G1	9.1 ± 5.03^{a}	$6.6 \pm 1.17^{ab*1}$	$0.5 \pm 0.17^{b*1}$	6.2 ± 4.32^{ab}	14.9 ± 4.73^{A}	$14.8 \pm 4.70^{A\#}$	$8.7 \pm 2.37^{A\#}$	9.1 ± 2.55^{A}
G2	12.4 ± 3.71^{ab}	14.6 ± 3.56^{a2}	7.4 ± 2.72^{ab2}	$5.9 \pm 3.42^{b*}$	15.7 ± 7.1010^{A}	11.4 ± 4.57^{A}	10.9 ± 5.56^{A}	$4.5 \pm 2.21^{A\#}$
RF (%)								
	Spring	Summer	Autumn	Winter	Spring	Summer	Autumn	Winter
G1	222.4 ± 121.84^{a1}	$20.6 \pm 6.81^{a*1}$	$0.0 \pm 0.00^{b*1}$	155.8 ± 144.88^{a1}	407.1 ± 70.27^{A1}	$421.1 \pm 119.19^{A\#1}$	$212.6 \pm 43.69^{A\#1}$	186.6 ± 59.37^{A1}
G2	2159.8 ± 705.14^{a2}	$145.6 \pm 42.94^{b*2}$	$148.6 \pm 60.74^{b*2}$	$1973.8 \pm 834.29^{c*2}$	2249.7 ± 750.68^{A2}	$1550.6 \pm 689.11^{A\#2}$	$1264.8 \pm 352.64^{A\#2}$	$2214.6 \pm 1235.70^{A\#2}$
E2 (pg/ml)								
	Spring	Summer	Autumn	Winter	Spring	Summer	Autumn	Winter
G1	$39.8 \pm 24.94^{ab*1}$	71.8 ± 40.73^{a1}	17.7 ± 1.18^{b1}	$40.1 \pm 13.74^{ab*1}$	$90.3 \pm 29.69^{A\#1}$	39.0 ± 14.44^{B1}	27.9 ± 17.20^{AB1}	$227.4 \pm 133.99^{C\#1}$
G2	1941.3 ± 233.00^{a2}	$942.0 \pm 237.51^{b*2}$	$1316.8 \pm 180.55^{ab*2}$	$1465.3 \pm 116.11^{ab*2}$	2252.6 ± 1145.64^{A2}	$3380.6 \pm 1462.29^{AB\#2}$	$4176.0 \pm 1370.58^{B\#2}$	$3486.1 \pm 1397.60^{AB\#2}$

[ab] Different letters indicate significant differences between seasons in the same group within the same environment (ab – wild; AB – captivity); [*#] Different symbols indicate significant differences between environment in the same season in the same group; [12] Different numbers indicate significant differences between groups in the same environment and season ($P < 0.05$)

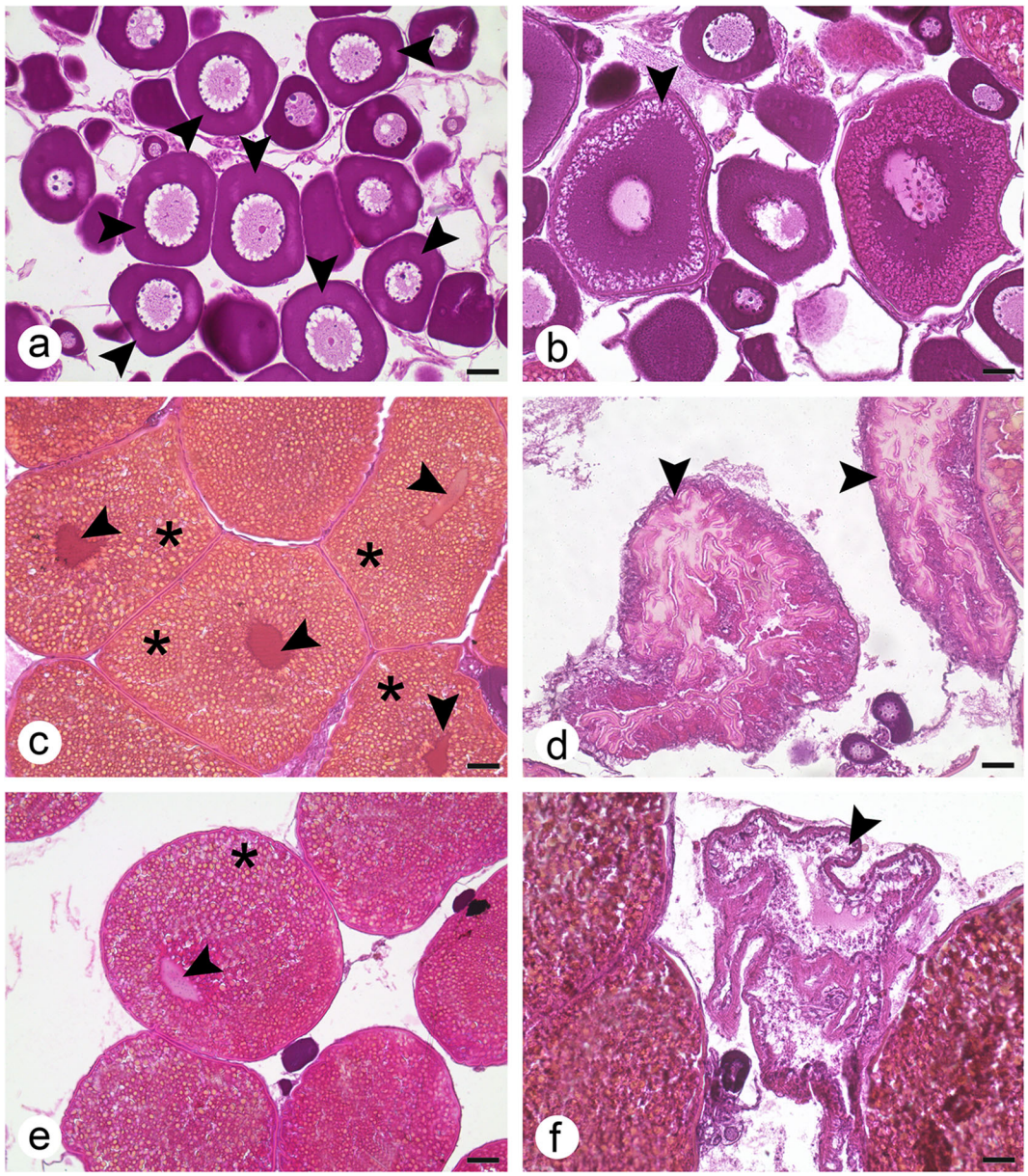

Fig. 1 *Astyanax fasciatus*. Oocyte developmental stages. PN: perinucleolar (**a**) (arrow head); CA: cortical alveoli (**b**) (arrowhead); V: vitellogenic (*) cytoplasm and nucleus (arrow head) (**c**); A: atretic (**d**) (arrow head); nuclear migration (arrow head) and vitellogenic (*) (**e**); POF: post-ovulatory follicle (**f**) (arrow head)

Relative fecundity (RF) and oocyte diameter

The frequency distribution of the oocyte diameters throughout the year was bimodal for all groups. The first component of the distribution (diameters ≤600 μm) corresponded to residual oocytes, i.e., the stock of oocytes to be recruited in the next breeding cycle. This peak comprised 60% to 70% of the oocytes in the G1 females (Figs. 4a and b) and 40% to 50% in the G2 females (Figs. 4c and d). The second component of the distribution included the oocytes with larger diameters (>600 μm) corresponding to vitellogenic oocytes and represented ~10% of the oocytes for all groups (Fig. 4a,d).

In all seasons, G1 females always had smaller RF values than G2 females ($P < 0.001$ in the spring and

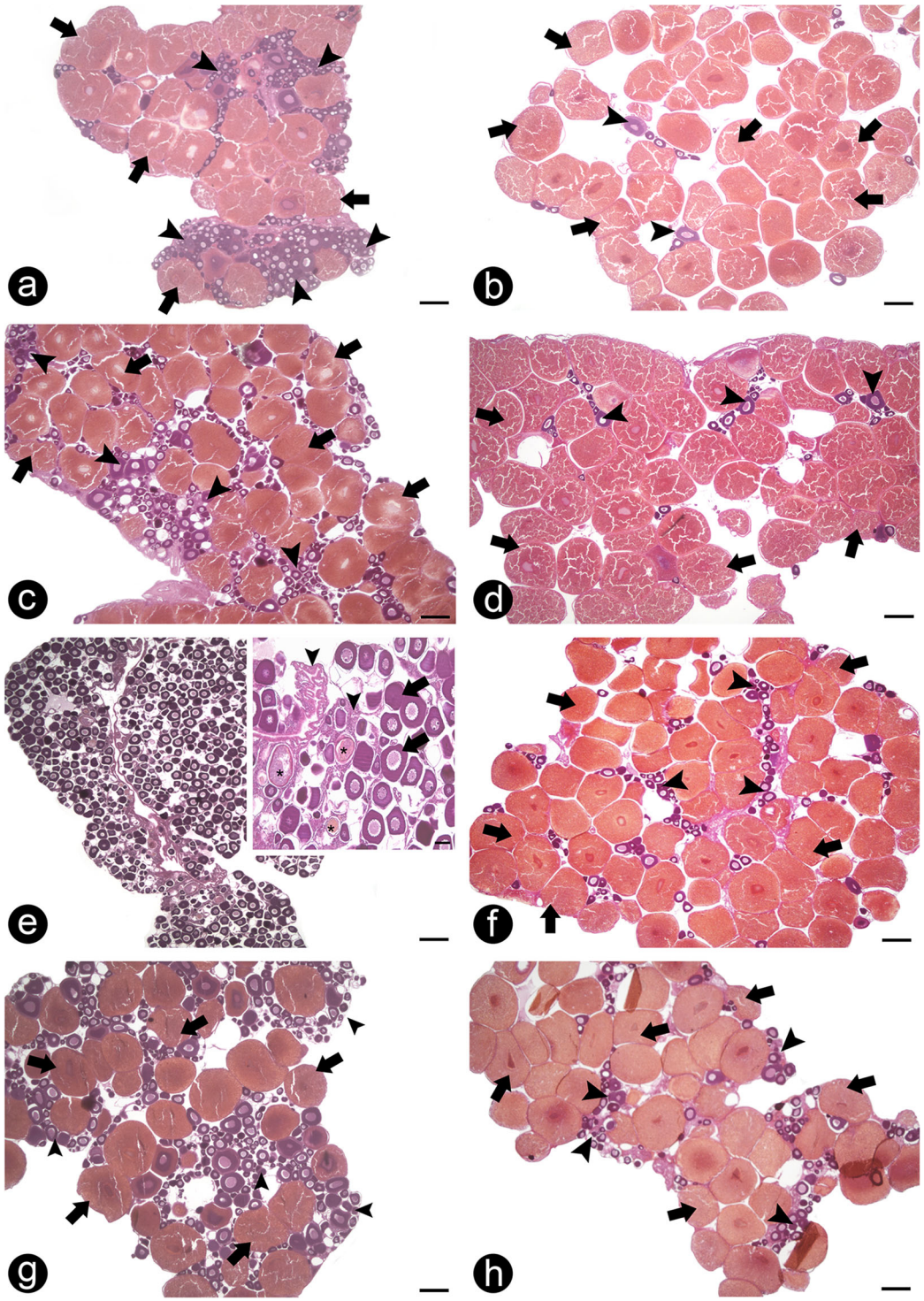

Fig. 2 Ovary sections of *A. fasciatus* with 46 chromosomes (G1) in the wild in spring (arrow: vitellogenic oocyte, arrow head: perinucleolar oocyte) (**a**); summer (arrow: vitellogenic oocyte, arrow head: perinucleolar oocyte) (**c**); autumn (zoon in insert - arrow: oocyte perinucleolar, arrow head post-ovulatory follicle, asterisk: atretic oocyte) (**e**); and winter (arrow: vitellogenic oocyte, arrow head: oocyte perinucleolar) (**g**). Ovary sections of *A. fasciatus* with 46 chromosomes (G1) in captivity in spring (arrow: vitellogenic oocyte, arrow head: oocyte cortical alveoli) (**b**), summer (**d**), autumn (**f**) and winter (**h**). In d, f and h -arrow: vitellogenic oocyte, arrow head: oocyte perinucleolar

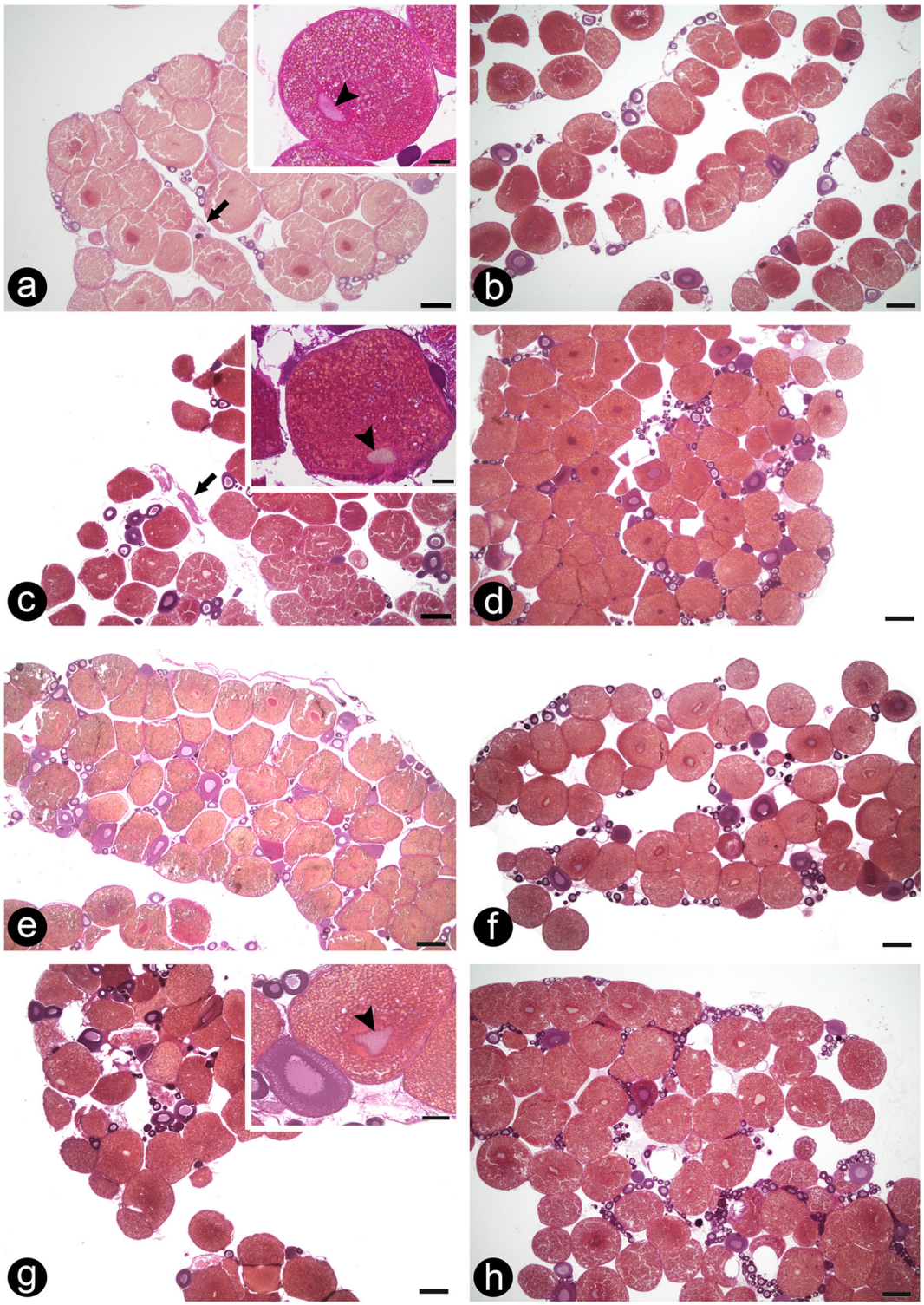

Fig. 3 Ovary sections of *A. fasciatus* with 48 chromosomes (G2) in the wild in spring (**a**) (arrow: post-ovulatory follicle), summer (**c**) (arrow: post-ovulatory follicle), autumn (**e**) and winter (**g**) (zoon in inserts a, b and g demonstrated the nuclear migration). Ovary sections of *A. fasciatus* with 48 chromosomes (G2) in captivity in spring (**b**), summer (**d**), autumn (**f**) and winter (**h**)

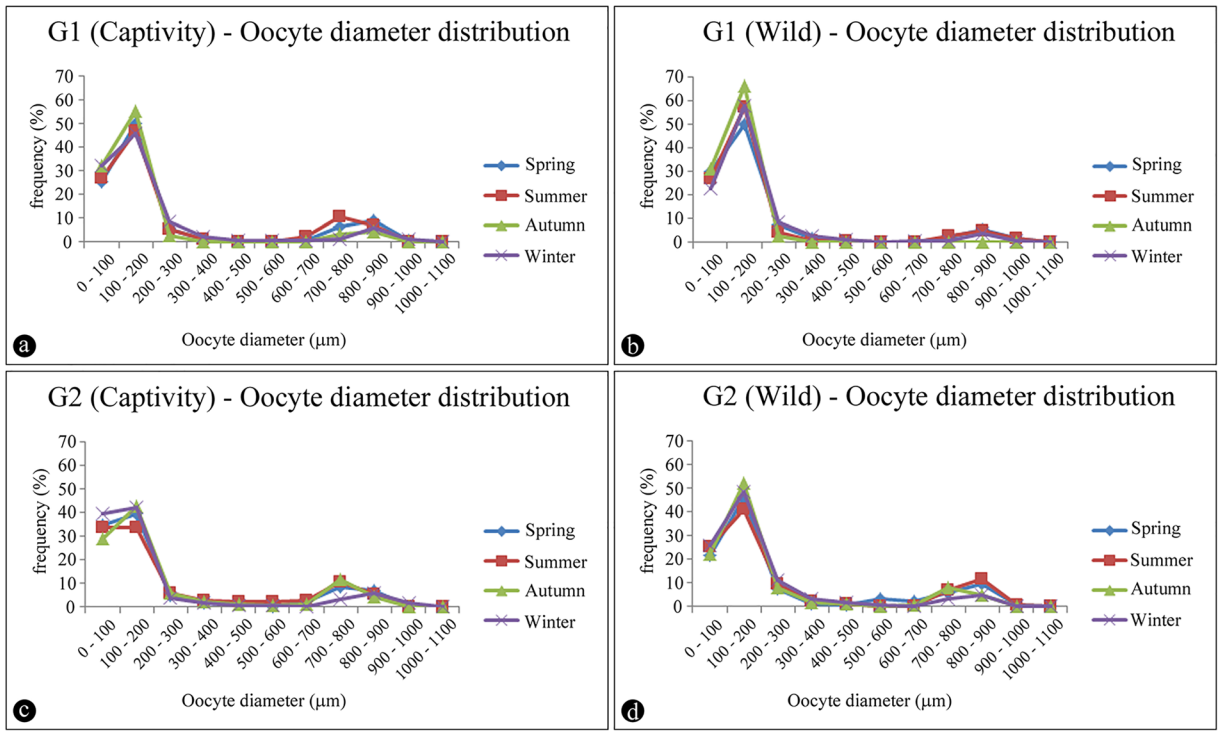

Fig. 4 Distribution frequency of oocyte diameters (mean) of *A. fasciatus* from G1 (2n = 46) in captivity (**a**), G1 in the wild (**b**), G2 (2n = 48) in captivity (**c**) and G2 in the wild (**d**). Different line colors indicate different season

winter and $P = 0.002$ in the autumn and summer). In the captive females, the G2 group had higher RF values than the G1 group during all seasons (spring, $P < 0.001$; summer, $P = 0.001$; autumn, $P = 0.002$; winter, $P < 0.001$; Table 1).

Vitellogenic oocytes with diameters greater than 600 µm were evaluated for *A. fasciatus* because at this diameter, they had a dense vitellogenic aspect. Wild G1 females showed a constant RF throughout the year, but no mature oocytes were found in the autumn, whereas the captive G1 females maintained a constant RF throughout all seasons. Compared with the captive females, the wild females had significantly higher RFs in the summer and autumn ($P < 0.001$ for both; Table 1).

The wild G2 animals showed similar RF patterns, but the absolute numbers differed from the G1 females. The RF of the wild G2 group was also high in the spring (2000 oocytes/g total body mass), although it significantly decreased in summer and autumn, reaching 200 oocytes/g total body mass. There was an increase in the winter, but it differed from the spring ($P > 0.001$). Captive females (G1 and G2) exhibited a different pattern, with a constant RF throughout the year. Significant differences were observed between the G2 females from the two environments during the summer, autumn, and winter, with higher RF values for the females in captivity ($P = 0.001$, $P = 0.007$, and $P < 0.000$, respectively; Table 1).

Plasma E2 concentration

The plasma E2 concentration in the wild G1 females remained almost constant throughout the year, except for autumn, when it was significantly lower than the summer ($P = 0.010$). In contrast, the captive G1 females presented a completely different hormonal pattern, with a decrease in E2 concentrations between the spring and summer ($P = 0.009$) that remained stable until autumn, and a significant, sharp increase in the winter relative to the other seasons (spring, $P = 0.008$; summer and autumn, $P < 0.001$). In the winter and spring, the captive females had higher E2 concentrations than the wild females (winter, $P = 0.001$; spring, $P < 0.001$; Table 1).

The plasma E2 levels in the wild G2 females were relatively constant throughout the year, with a significant decrease observed only between the spring and summer ($P = 0.006$). Relative to wild G2 females, there was no change in the pattern for the captive G2 group,

and the greatest difference in the E2 concentrations was between the autumn and spring (P = 0.009). The captive G2 females also had higher E2 concentrations than the wild females during the summer, autumn, and spring (P = 0.001, $P < 0.002$ and P < 0.002, respectively; Table 1). Comparing the G1 and G2 groups revealed that both the wild and captive G2 females had higher E2 levels than the G1 females ($P \leq 0.001$; Table 1).

Discussion

Interactions between the environment and the genetic, physiological, behavioral, and ecological responses of individuals are related to the reproductive tactics of the species (Taborsky et al. 2008). In its natural environment, *A. fasciatus* is known to have multiple spawnings in the same reproductive period (Gurgel 2004).

Astyanax is a very diverse genus spread across the neotropical countries, occurring from the southern United States to Argentina. Karyotypic variations occur in *A. bimaculatus*, *A. scabripinnis*, and *A. fasciatus*, suggesting the existence of species complexes (Pansonato-Alves et al. 2013; Rossini et al. 2016). However, Ferreira-Neto et al. (2012) proposed that the 2n = 46, 2n = 48, and 2n = 50 *A.* cf. *fasciatus* karyomorphs are distinguished species (sensu biological species concept). Therefore, the mechanisms whereby such karyotypic differentiation results from speciation are still moot. Our study suggests that the marked differences in the reproductive cycles between the two sympatric karyotypes may be one of the factors leading them to species differentiation.

The results of this study showed that wild G1 females start their reproductive cycle in winter and spawn in autumn, while wild G2 females begin their reproductive cycle in autumn and show a spawning peak during the spring and summer. However, in captivity, both fish cytotypes showed changes in their reproductive cycles relative to those in the wild. During the reproductive cycle, the increase in ovary mass is primarily characterized by the uptake of vitellogenin produced in the liver in response to increasing E2 levels in the plasma (Reading et al. 2017). In addition, a close relationship exists between the gonadal maturation process and the increasing volume and mass of the ovaries. Thus, the GSI is an important quantitative parameter because its temporal pattern provides information about the reproductive period of the species (Flores et al. 2019).

The difficulty in inducing spawning in captive migratory animals is well documented (Zohar and Mylonas 2001). In this study, for example, the wild G1 females apparently maintained all studied reproductive parameters at high levels throughout most of the year (RF, GSI, and ovarian morphology) due to the observed high E2 concentrations. The exception was in the autumn, when these indices decreased, and POFs were observed, indicating the likely spawning season for these animals. This indicates that the animals adjusted to captivity by continuing to produce vitellogenic oocytes, with both temporal and quantitative changes seen in the reproductive parameters studied. This profile suggests that the smaller energetic requirements in captivity allow for the maintenance of higher fecundity, as well as estradiol levels.

Honji et al. (2009) evaluated the fecundity of wild and captive female *Salminus hilarii* (another characid fish), which also has an established spawning protocol (Honji et al. 2011). They reported that the captive animals had a higher percentage of vitellogenic oocytes than those from the rivers. They also observed a lower fecundity in the captive animals even though these females had mature oocytes with larger diameters, thus demonstrating a difference between the two environments. In an additional study in *S. hilarii*, Moreira et al. (2015) showed that these changes are explained by the reduced gene expression of luteinizing hormone (LH) and the follicle-stimulating hormone (FSH) β subunit, and by lower plasma E2 levels in captive females relative to those in the wild. Guzman et al. (2009) also reported on differences between environments in an analysis of the gene expression of gonadotropin-releasing hormone (GnRH), FSH, and LH in *Solea senegalensis* females, with wild females having higher values than captive females.

The RF and GSI data and the absence of POFs in this study demonstrated that captive G1 *A. fasciatus* females do not spawn, as has previously been described for other potamodromous migrating species such as *S. hilarii* (Honji et al. 2009), *Prochilodus lineatus* (Hainfellner et al. 2012a), and *Brycon cephalus* (Hainfellner et al. 2012b). Based on this information, the induced breeding of G1 animals in captivity should have better outcomes between winter and spring, the period immediately following peak estradiol production with its consequent increases in RF, GSI, and vitellogenic oocytes.

The wild G2 females began their reproductive cycle earlier than the wild G1 females, showing a gradual

increase in E2 concentrations during the autumn that then peaked in the spring, accompanied by an increase in RF. These parameters decreased in the spring and summer, with spawning detected by the presence of POFs. In some tropical animals, the spawning period is linked to the rainy season (from spring to late summer), a period with an increased frequency and intensity of precipitation combined with high water temperatures, which provide a suitable environment for the survival of offspring (Andrade and Braga 2005). In other words, in the wild, G2 females appear to be seasonally advanced relative to G1 females. However, the morphology of the ovaries showed that these females retained vitellogenic oocytes throughout the year, with POFs present in spring and summer, whereas G1 females had POFs in the autumn without vitellogenic oocytes. These results may represent a tactic (Taborsky et al. 2008) used by this cytotype to vary the total number of vitellogenic oocytes throughout the year. However, ovaries always have a certain number of these oocytes, i.e., the total number of oocytes decreases throughout the year, but they remain vitellogenic.

When G2 animals were held in captivity, the analyzed parameters remained unchanged relative to the G1 animals. These females had ovaries in the advanced-maturation stage throughout the year but without evidence of POFs, demonstrating that they did not spawn in captivity, which is consistent with the results from studies on other characiforms (Hilsdorf et al. 2014). Captive fish apparently alter their sensitivity to environmental cues regarding the seasonal cycle, and their E2 levels remain higher throughout the year. This constitutes an adjustment of the set point for the variable and may explain the greater success in inducing some species of characiforms to spawn in captivity.

Zohar and Mylonas (2001) have proposed the existence of three different types of reproductive dysfunction in teleost females held in captivity. The first type of dysfunction is a complete failure of vitellogenesis, which is common in eels. The second and most common type of dysfunction is an absence of the final maturation of oocytes. Vitellogenesis appears to progress normally, but germinal vesicle breakdown (GVBD) and ovulation do not occur. This is typical for most potamodromous teleost species. The third type of dysfunction, which is common in trout, is when the final maturation and ovulation phases occur but spawning does not. In the latter two types, the oocytes are usually reabsorbed. Both groups in this study (G1 and G2) exhibited maturation with an apparent lack GVBD. Therefore, they demonstrate the second type of dysfunction.

The data from this study revealed that the reproductive biology of the G1 and G2 cytotype females in the *A. fasciatus* group differ from each other, even in the wild. They also differed from previous data collected on this fish (Gurgel 2004; Silva et al. 2010). However, the RF data showed similar temporal reproductive profiles for the two groups in the wild, with higher values in winter and spring, but with different absolute values. According to Gurgel (2004), who studied the reproduction of *A. fasciatus* in the Rio Grande do Norte (Brazil), spawning is synchronous in groups, with greater reproductive activity in October/November and December/January. However, Silva et al. (2010), who conducted studies in Paraná (Brazil) on the same species, reported synchronous spawning at the Iraí Dam and asynchronous spawning at the Piraquara and Passaúna dams. Thus, it is difficult to establish a standard reproductive cycle for the species, which apparently has significant reproductive plasticity depending on its environment. Similar observations have been made in *H. malabaricus*, which also responds with reproductive plasticity depending on reservoir water quality (Gomes et al. 2015). In this study, the animals showed synchronous spawning within each group. It is also clear that the reproductive variables for the G1 and G2 cytotypes had completely different absolute values. For example, the RF peaked at 400 in the G1 females, but reached 2500 vitellogenic oocytes in the G2 females (adjusted for body mass). The estradiol levels also differed between the groups, with a maximum of 250 pg/mL in G1 females and ~4000 pg/mL in G2 females, substantiating the length data, which also showed a clear difference between groups.

The data presented here support the conclusion that G1 females (2n = 46) maintain a seasonal reproductive cycle in captivity, but that this cycle differs from that observed in the wild. Thus, induced breeding practices have a reasonable probability of success when the females are handled soon after the vitellogenic period begins (identified in this study as occurring during the winter) to induce ovulation and spawning. In addition, G2 females (2n = 48) in captivity appear to possess a high degree of reproductive plasticity. Even in the absence of seasonal synchrony with the natural environment, the ovaries remained vitellogenic and prepared for induced spawning throughout most of the year. The same distinct reproductive patterns were observed in

captivity, which implies a need for developing specific induced-spawning protocols for each cytotype. Lastly, the reproductive differences found between the two sympatric *Astyanax* groups suggest the two cytotypes might have been maintained to distinguish their reproductive timing in the wild. These findings are important considering the physiological basis has now been found that is consistent with the specific characteristics of the distinctive reproductive tactics of *A. fasciatus*.

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