Factors affecting secondary sex characteristics in the yellowtail tetra, *Astyanax altiparanae*

Diógenes H. Siqueira-Silva · Rafaela M. Bertolini · Nycolas Levy-Pereira · Nivaldo F. Nascimento · José A. Senhorini · Lucas Henrique Piva · José Bento S. Ferraz · George S. Yasui

Received: 5 November 2019 / Accepted: 30 May 2020 / Published online: 19 June 2020
© Springer Nature B.V. 2020

Abstract This study interrogated factors which affect the appearance of secondary sexual characteristics, namely, fin spinelets (rigid dimorphic structure empirically associated with male sexual maturity in characids), in *Astyanax altiparanae*. Many variables such as the season of the year and several biotic components, including organism length, sex, phase of maturation, and the presence of gonads, were investigated. These factors were then associated with the physiological development of fin spinelets. The development of this trait is related to reproductive strategies but demonstrates considerable population variability as it is found throughout the year in some species but only during specific periods in others. Seventy-five specimens obtained from spontaneous spawn of farmed fish were arbitrarily grouped into small-, medium-, and large-sized groups in both summer and winter. Gonadal histology was performed to confirm each animal’s sex and phase of maturation. Diaphanization of the fish was performed to visualize, count, and measure the fin spinelets. Finally, gonadectomization of some males was utilized to investigate the gonadal effect on the presence of fin spinelets. The present results show that the presence of fin spinelets is a secondary sexual characteristic of males which occurs independently of the season and is always present in males longer than 48 mm. However, in the summer, male specimens presented more rays with fin spinelets than during the winter. Furthermore, since fin spinelets were observed on immature males as well as spawning capable males, their presence cannot be directly associated with sexual maturity in male *A. altiparanae*, as previously supposed. Finally, gonadectomization resulted in an initial reduction in the length of fin spinelets. However, this trend was eventually normalized with time.

Keywords Diaphanization · Fish reproduction · Gonadectomy · Spinelets

Introduction

Most teleost fish spawn are seasonal events that depend on a series of environmental conditions required to
trigger reproductive migration and subsequent gonadal maturation. Since longer photoperiods, increased rainfall, and higher temperatures improve food availability for offspring, parental animals usually become spawning capable during the summer season (Baldisserotto 2013). However, some species, such the yellowtail tetra Astyanax altiparanae (Garutti and Britski 2000), demonstrate peak reproduction during the spring and summer, but also participate in intertidal spawning, and may reproduce several times throughout the year, including during the winter season (Rodrigues et al. 1992; Sato et al. 2006).

In males of that species, small bony hooks called fin spinelets (rigid dimorphic structure) can be observed on the anal fins and are usually associated with male sexual maturity (DO Nascimento et al. 2017). The fin spinelets are observed in several species from the Characiform order, including the tetra Deuterodon stigmatus (Dalcorte and Fialho 2013), and other important aquaculture fish, such Salminus brasiliensis and Brycon orbignyanus (Veríssimo-Silveira et al. 2006). Since fin spinelets are always present in mature males during the reproductive period, they are believed to be used to adhere to the anal fin of females during spawning (Malabarba and Weitzman 2003). In addition, they are believed to be a sexual dimorphism that can influence reproduction and indicate the beginning of the reproductive period. Azevedo et al. (2000) hypothesize they are related to female courtship behavior, and DO Nascimento et al. (2017) believe they can stimulate female spawning. Other types of secondary sexual characters are observed in fish. In the Amazonian peacock bass from the genus Cichla, males develop a post-occipital protuberance during the reproductive period (Kelber 1999). This structure is composed primarily of lipids, which are thought to provide energy during the parental care period (Jepsen et al. 1999; Muñoz et al. 2006).

Such dimorphic structures are important to aquaculture, since they can be used in management and sex identification during artificial insemination. They are also relied upon to confirm the status of gonadal maturation, as stated in several studies (Porto-Foresti et al. 2005; Bem et al. 2012; DO Nascimento et al. 2017) for A. altiparanae reproduction in captivity. Although the development of anal-fin spinelets is correlated with A. altiparanae sexual maturity (Porto-Foresti et al. 2005; Nascimento et al. 2017), there is no direct evidence that the presence of fin spinelets indicates gonadal maturation in this Characiform species.

The objective of this study was to understand the onset, developing, and maintenance of the fin spinelets in A. altiparanae according to maturation phases during winter and summer seasons, with the purpose of improving management of this species in aquaculture. This is pertinent due to the fact that A. altiparanae’s role in fish farming has grown significantly in recent years, with data from 2016 to 2017 illustrating an increase of 136.07%, making it the fifth most important species by production quantity in Brazil (IBGE, 2017). This scenario is largely due to the yellowtail tetra’s desirable livestock characteristics, such as ease of handling, small size, early sexual maturity, and intertidal spawning (Rodrigues et al. 1992; Sato et al. 2006). In addition, yellowtail tetra are widely consumed by the human population, as food in the form of appetizers, as bait in sport fishing (Fonseca et al. 2017), and as a promising candidate in canned fish markets (Porto-Foresti et al. 2005). Apart from its importance in aquaculture, the choice of A. altiparanae was motivated by its increasing utility as a biological model, as evidenced by the diversity of studies published using it. Therefore, the research evaluated how the following factors affect the development of A. altiparanae: the presence, length, and maximum number of rays with fin spinelets in accordance with the gender and length of the animals during both winter and summer. Lastly, the effects of gonadectomization on those parameters of fin spinelets were also studied.

Materials and methods

Origin of broodstock and gamete sampling

All the procedures were performed in accordance with the Ethical Committee for the Care and Use of Laboratory Animals at Chico Mendes Institute (CEUA-CEPTA #02031.000033/2015-11).

The yellowtail tetra (A. altiparanae) were collected from the Mogi Guassu River (21.925706° S, 47.369496° W) and maintained in 1000 m² earthen ponds (~500 fish per pond, SL ≈ 12 cm). As this species spawns spontaneously, F1 offspring were produced within a few months. These F1 fish were selected for participation in the following experiments.
Experiment I: secondary sex characteristics in winter and summer seasons

This experiment was divided into two trials. The first trial was performed during the winter (July 2013; air temperature, 16.67 °C; water temperature, 18 °C; dissolved oxygen, 6.73 mg/L) and the second trial during the summer (January 2014; air temperature, 25.67 °C; water temperature, 29 °C; dissolved oxygen, 4.43 mg/L). In each period, 75 adult specimens, ranging from 6 to 12 months old, were collected using fishing nets (4.75 × 1.10 m) with 1.0 × 1.0 mm mesh from one earthen pond. Fish were divided into three groups of 25 animals each according to their body length. Initially, the fishes were visually separated by our own observation criteria, with no use of measurement scale. Then, for the measurement process, the fish were anesthetized in menthol solution (100 mg.L⁻¹) before having their standard length (mm), height (mm), and weight (g) recorded. The fish were then sorted into three groups as follows: small (45.85 ± 5.39 mm), medium (60.54 ± 5.89 mm), and large (80.72 ± 7.08 mm).

Histological analysis of the gonads

To define the sex and maturation status of the collected specimens, fish were euthanized using 2-phenoxyethanol (C₆H₁₀O₂ – SIGMA-ALDRICH). Their gonads were removed, cut into transverse and longitudinal sections, and fixed with Bouin’s fixative (Adria laboratories, Londrina, Brazil) for 24 h. Samples were dehydrated in a series of graded ethanol solutions, embedded in paraffin – polysisobutylene mixture (Paraplast®, Sigma-Aldrich), sectioned at 5.0 μm on a microtome (Leica RM2235, Wetzlar, Germany) equipped with steel blade (Leica 818), and finally stained with hematoxylin and eosin. All samples were examined on a microscope (Nikon SMZ 1500, Nikon, Tokyo, Japan), and digital images were captured using a CCD camera (Nikon DSF1, Nikon, Tokyo, Japan). The images were then analyzed with NIS-Elements AR software (Nikon, Tokyo, Japan).

Morphological analysis of diaphanized fins rays

In order to determine the number and length of fin rays, as well as the presence, number, and length of fin spinelets, each animal was fixed in 5% formalin for 24 h before being submitted to diaphanization using the Potthoff (1984) protocol. Samples were washed in distilled water for 24 h and then dehydrated in ethanol solutions (50% for 24 h and 95% for 24 h). For cartilage staining, the samples were incubated in Alcian blue acidified ethanol solution (20 mg of Alcian blue 200 mg.L⁻¹, 60% of ethanol, 40% of glacial acetic acid) for 24 h. After this, the samples were incubated in saturated borate solution for 5 h and then sequentially treated in whitening solution (3% of H₂O₂ and 2% of KOH, 2 h) and clarifying solution (35% saturated borate solution and 65% of the whitening solution, 7 h). For bone staining, the samples were incubated in Alizarin solution (Alizarin 2% and KOH 2% in distilled water, for 24 h) before being transferred to successive preservation solutions (solution I, glycerin 30% and 70% of KOH 2% solution for 24 h; solution II, glycerin 60% and 40% of KOH 2% solution). Finally, the samples were kept in maintenance solution (100% glycerin with thymol crystals).

Experiment II: effects of gonadectomy on secondary sex characteristics

Long-term anesthesia for surgical procedures

Prior to surgery, fish were placed in 2-L beakers containing 1 L of 0.7% 2-phenoxyethanol solution in tap water. The following anesthetic parameters were then assessed: hyperactivity, erratic swimming, non-physiologic positioning, and absence of opercular ventilation.

After the induction of anesthesia, fish were transferred to a surgical apparatus as described by Harms and Lewbart (2000). Briefly, the apparatus was composed of a 6 L-aquarium containing anesthetic solution and a pump (4 mL.s⁻¹) coupled to a silicon cannula (3.5-mm internal diameters and 4.5-mm external diameter).
The fish were placed in dorsal decubitus on a sponge cut into a “V” tray supported by a net placed on the water surface. The cannula was inserted into the oral cavity of the fish in a way that maintained solution flow from the gills through the operculum.

Twenty-four fish were divided into six treatment groups ($n = 4$): For T1, the control treatment, a solution free of any anesthetic was used. For T2, T3, T4, T5, and T6, solutions containing 0.1, 0.2, 0.3, 0.4, and 0.5% of 2-phenoxyethanol were used. For each treatment, the period of anesthesia induction was registered, from the cessation of operculum movement until the return of caudal reflexes. As a reference, 60 min was adopted as the maximum period of anesthesia, in which it is possible to perform surgical interventions of long duration (Yasui et al. 2009).

**Gonadectomy**

Before surgery the fish underwent fasting for 24 h in order to empty their gastrointestinal system. During the surgery, the fish were anesthetized and positioned in surgical beds as previously described. As determined by the previous experiment, a maintenance anesthetic solution containing 0.5% of 2-phenoxyethanol was used.

Twenty eight adult male specimens, presenting fin spinelets, evident to touch, were separated into two groups ($n = 14$). In group 1 (control), the fish were incised and then sutured. In group 2, the gonadectomy was performed by a ventral incision made from the pectoral fins to the posterior region of the pelvic fins. The coelomic cavity was explored in order to find the gonads, which were then extracted with a hemostatic clamp. The coelomic cavity was sutured with two separated single stitches using a class II-monofilamentary wire (nylon 4-0, Shalon fios cirúrgicos LTDA., Brazil), with an approximated distance of 9 mm (Yasui et al. 2009).

To standardize the surgical duration in both treatments, the non-gonadectomized fish were left in the surgical bed during the same time as the gonadectomized fish. This experiment was performed during summer because the fish with fin spinelets were more numerous and larger than other periods.

After surgery, the fish were allocated to a 416-L aquarium, free from light (covered in aluminum foil), with water containing 2 g of Aurotrim (chlortetracycline 1.5%, sulfadiazine 7.5%, and trimethoprim 1.5%) per 1000 L. The fish were deprived of food for 96 h. The stitches were removed 4 days post-surgery (4 dps). Thirty days post-surgery, the fish were captured, euthanized with anesthesia, and submitted to diaphanization as previously described.

The effect of the gonadectomy on fin spinelets formation was analyzed at either 30 days post-surgery or 90 days post-surgery. For each time point, seven fish from each experimental group were diaphanized according to the protocol previously described.

### Statistics

All data are reported as the mean ± the standard deviation. The data were tested for normality using a Lilliefors test. In the case of normally distributed data, analysis was performed using ANOVA followed by Tukey’s multiple range test. For the comparison of two means (e.g., winter and summer, gonadectomized or non-gonadectomized), a non-paired t-test was used. In all cases, a type I error threshold of 0.05 was adopted.

### Results

**Experiment I**

**Histological analysis of the gonads**

**Winter trial** Most of the specimens categorized as small were male. The majority of fish were in the developing stage (eleven in initial maturation phase and nine in mid-maturation phase) (Figs. 1 and 2). Females from this group were beginning the developing phase (Figs. 1 and 3). One individual could not be identified. Most of medium-sized fish were female. There were immature, developing, and spawning capable animals (Figs. 1, 2, and 3). All the male specimens of this size were in the developing phase, with three in the initial maturation and six in the mid-maturation (Figs. 1 and 2). Of the fish classified as large, 23 were female, with most in developing and spawning capable phases (Figs. 1 and 3). Both of the males from this group were in the developing phase (Figs. 1 and 2).

**Summer trial** In both the small- and medium-size categories, most of the specimens were male (Fig. 1). Among small-sized fish, the majority were in the developing phase. Among the males of this group, five were
in the initial maturation phase, and eleven were in the mid-maturation phase. Most of the medium-sized males were also in the mid-maturation phase (Figs. 1 and 2). All the females were spawning capable (Figs. 1 and 3). Among large-sized specimens, most were female. Of these fish, most were spawning capable (Figs. 1 and 3).

Morphological analysis of the diaphanized fins rays In the small-sized group, those fin spinelets were present in fewer rays and had decreased lengths, and fish gonadosomatic index (GSI) lowered score when compared with fish captured in summer (Table 1). In the other two groups, most of the analyzed variables were statistically similar in the fishes between the seasons. Only both, the GSI values and rays with spinelets, in the medium-sized group, were lower in the animals sampled in the winter than the ones from summer (Table 1). In winter, 29 out of 33 (87.88%) captured males presented fin spinelets (all the animals with no fin spinelets were from small-sized-animals), while all 41 (100%) males captured during the summer exhibited fin spinelets (Fig. 4).
One female captured in summer presented fin spinelets, with a lower number and distribution when compared with males. No female captured during the winter had spinelets (Table 2).

The observed, incremental difference in average GSI from winter to summer was likely due to the reproductive peak achieved during the latter period (Fig. 1; Table 2). However, the females from the small-sized group exhibited little variation, which is related to only one spawning capable female in the summer season (Fig. 1; Table 2).

Experiment II

Long-term anesthesia for surgical procedures

Doses of 0.1, 0.2, and 0.3% 2-phenoxyethanol were not effective, with just 0.78, 1.70, and 2.50 min of anesthesia, respectively. Those results were similar to that observed in the control group (0.0% 2-phenoxyethanol) with 0.90 min of anesthesia ($P < 0.0001$). On the contrary, with a dose of 0.4% 2-phenoxyethanol, the time of anesthesia increased significantly ($P = 0.0001$) to 35.40 min, with one individual reaching 60 min. However, the dosage of 0.5% 2-phenoxyethanol was sufficient for all fish to remain in anesthesia for 60 min while also providing adequate time for the relevant surgical procedures.

Gonadectomy

Thirty days after surgery, no differences were observed between the control and gonadectomized fish for number of spinelets ($P = 0.1122$; 70.20 ± 9.00 and 50.70 ± 8.50, respectively) (Table 3)

Fig. 2  Histology characteristics of *A. altiparanae* testes phases. **a** Immature (inset: highlighting spermatogonia (arrow), which are very characteristic of this phase). **b** Developing (Z = sperm). **c** Spawning capable (inset: showing discontinuous epithelium (arrow head)). **d** Regressing (spermatogonia (arrow), RZ = residual spermatozoa). Scale bars, a–d 100 mm; insets, 25 μm

Fig. 3  Histology characteristics of *A. altiparanae* ovaries phases. **a** Immature, showing only oocytes with perinucleolar nucleus; **b** developing, showing pre-vitellogenic oocytes with perinuclear nucleolus (PnO) and oocytes with cortical alveoli (CaO); **c** spawning capable; with vitellogenic oocytes. Scale bars: a–c 100 μm
and rays with spinelets ($P = 0.7115$; $10.00 \pm 1.00$ and $9.20 \pm 1.50$, respectively) (Table 3). However, a significant decrease ($P = 0.0478$) in the length of spinelets was observed for gonadectomized fish ($90.82 \pm 7.04 \mu m$) when compared with the control group ($114.75 \pm 8.26 \mu m$) (Table 3).

Table 1 Meristic and somatic parameters of *Astyanax altiparanae* male in winter 2013 and summer 2014

| Season | Size classes | Number of animals ($n$) | Weight (g) | Standard length (mm) | GSI (%) | Spinelet presence (%) | Spinelet length ($\mu m$) | Total of spinelets | Rays with spinelets* |
|--------|--------------|-------------------------|------------|-----------------------|---------|------------------------|--------------------------|---------------------|---------------------|
| Winter | Small        | 22                      | 2.79 ± 0.73 | 43.84 ± 3.58          | 1.01 ± 0.53a | 82                    | 26.00 ± 37.20a          | 26.47 ± 19.15a       | 11                  |
|        | Medium       | 9                       | 6.15 ± 2.12 | 55.75 ± 6.92          | 1.29 ± 0.70b | 100                   | 38.49 ± 55.87b          | 57.62 ± 22.95b       | 12                  |
|        | Large        | 2                       | 7.35        | 59.95                 | 0.74ab    | 100                   | 15.00 ± 29.46ab         | 47ab                 | 9                   |
| Summer | Small        | 20                      | 3.43 ± 1.00 | 50.24 ± 5.44          | 2.50 ± 1.30b | 100                   | 67.20 ± 34.21b          | 100.06 ± 55.51a      | 29                  |
|        | Medium       | 20                      | 6.39 ± 1.41 | 62.52 ± 3.89          | 2.79 ± 0.65b | 100                   | 66.40 ± 52.64b          | 85.68 ± 27.97b       | 29                  |
|        | Large        | 1                       | 13.07       | 78.36                 | 2.2ab     | 100                   | 49.03 ± 78.49b          | 97ab                 | 9                   |

Means followed by different letters in the same column differ statistically

*These data refers to the maximum number of ray with spinelets

and rays with spinelets ($P = 0.7115$; $10.00 \pm 1.00$ and $9.20 \pm 1.50$, respectively) (Table 3). However, a significant decrease ($P = 0.0478$) in the length of spinelets was observed for gonadectomized fish ($90.82 \pm 7.04 \mu m$) when compared with the control group ($114.75 \pm 8.26 \mu m$) (Table 3).

Ninety days after surgery, no differences were observed among control and gonadectomized fish for number of spinelets ($P = 0.6138$; $79.30 \pm 8.60$ and $71.10 \pm 13.10$, respectively), rays with spinelets ($P = 0.3554$; $7.70 \pm 0.70$ and $6.40 \pm 1.10$, respectively), and length of spinelets ($P = 0.2705$; $127.70 \pm 7.74$ and $104.11 \pm 74.3$) (Table 3).

Fig. 4 Fish diaphanized according to Potthoff (1984). Lateral view of *A. altiparanae* (a); A diaphanized anal fin of a male presenting fin spinelets (arrows) (c–d). Detail of fin spinelets (arrow). Scale bars: b, 500 $\mu m$; c–d, 300 $\mu m$
Discussion

Sexual dimorphic structures tend to appear during the breeding season. One example is the hook that develops on the lower jaw along with enlarged teeth in male Atlantic salmon (*Salmo salar*). These traits are used to perform quiverings and yawnings, which seem to positively influence reproductive outcomes (Järvi 1990). Sometimes sexual dimorphic structures are a permanent characteristic, as in *Betta splendens* (Faria et al. 2007). In this species, males display a brightly colored body pattern and are bigger than females. These characteristics play an important role in ensuring reproductive success and can be supplemented by courtship behavior and parental care. The present results suggest that the anal-fin spinelets in *A. altiparanae* are a permanent dimorphic characteristic associated with adult males. This is evident based on the fact that these structures were observed in male specimens of all maturity phases. However, three fishes presenting testis in developing phase in the winter and one immature animal in the summer did not present fin spinelets. Do Nascimento et al. (2017) observed no fish with fin spinelets 52 days after hatching. This corroborates the lack of data associating these structures with juveniles, as observed for other Characiformes species (Lampert et al. 2004; Gonçalves et al. 2005; Dala-Corte and Fialho, 2014).

This study also determined that the presence of fin spinelets alone in *A. altiparanae* does not indicate that the males are able to reproduce. This was empirically presumed and adopted by authors during experimentation, mainly in hypophysation, where “spawning capable males” are selected for reproductive purposes. In Chehade et al. (2015), a similar conclusion was drawn, since males selected for by the presence of fin spinelets exhibited testicles containing cysts of all germ cells and 18.89, respectively) (Table 3). One fish in the gonadectomized group presented no anal-fin spinelets.

### Table 2  Meristic and Somatic Parameters of *Astyanax altiparanae* Female in Winter 2013 and Summer 2014

| Season | Size Classes | Number of Animals (*n*) | Weight (g) ± Standard Deviation | Standard Length (mm) ± Standard Deviation | GSI (%) ± Standard Deviation | Spinelet Presence (%) | Spinelet Length (μm) ± Standard Deviation | Total of Spinelets | Rays with Spinelets |
|--------|--------------|--------------------------|--------------------------------|------------------------------------------|-----------------------------|----------------------|------------------------------------------|-------------------|---------------------|
| Winter | Small        | 2                        | 3.82 ± 0.58 ± 0.00          | 46.66 ± 5.10 ± 0.00                      | 2.52 ± 1.38 ± 0.00         | 0                    | -                                        | -                 | -                   |
|        | Medium       | 16                       | 7.27 ± 1.76 ± 0.00          | 58.71 ± 6.02 ± 0.00                      | 3.20 ± 1.60 ± 0.00         | 0                    | -                                        | -                 | -                   |
|        | Large        | 23                       | 17.67 ± 4.54 ± 0.00         | 78.99 ± 7.18 ± 0.00                      | 3.20 ± 1.80 ± 0.00         | 0                    | -                                        | -                 | -                   |
| Summer | Small        | 5                        | 2.2 ± 0.66 ± 0.00           | 42.08 ± 3.93 ± 0.00                    | 1.60 ± 3.90 ± 0.00         | 0                    | -                                        | -                 | -                   |
|        | Medium       | 5                        | 8.41 ± 0.96 ± 0.00          | 65.54 ± 3.18 ± 0.00                    | 17.12 ± 4.67 ± 0.00        | 0                    | -                                        | -                 | -                   |
|        | Large        | 24                       | 17.82 ± 3.28 ± 0.00         | 83.34 ± 5.11 ± 0.00                    | 15.50 ± 7.5 ± 0.00         | 4.17                  | 117.89 ± 64.37 ± 0.00                      | 29                | 5                   |

Means followed by different letters in the same column differ statistically

*These data refers to the maximum number of ray with spinelets

### Table 3  Characterization of Fin Spinelets in Gonadectomized and Not Gonadectomized Males of *A. altiparanae*

| Days post-surgery | Control | Gonadectomized | *P*-value | Control | Gonadectomized | *P*-value |
|-------------------|---------|----------------|-----------|---------|----------------|-----------|
| Treatments        |         |                |           |         |                |           |
| Number of Spinelets (*n*) | 70.2 ± 9.0 | 50.7 ± 8.5 | 0.0122   | 79.3 ± 8.6 | 71.1 ± 13.1 | 0.06138   |
| Rays with Spinelets (*n*) | 10.0 ± 1.0 | 9.2 ± 1.5 | 0.07115 | 7.7 ± 0.7 | 6.4 ± 1.1 | 0.03554 |
| Spinelet Length (μm) | 114.75 ± 8.26 | 90.82 ± 7.04 | **p** | 127.70 ± 7.74 | 104.11 ± 18.89 | 0.02705 |

Means followed by different letters in the same line differ statistically
were not considered to be in the actively spawning capable phase. As stated by Costa et al. (2014), testes of the same specimen in *A. altiparanae* species can show different phases of the germinal epithelium maturation, named by De Siqueira-Silva et al. (2017) as maturation “waves,” misleading the real maturation phases of the individuals. This feature may be a male strategy to ensure success reproduction in any period of the year, since, as inferred by Cassel et al. (2017), female specimens are spawning capable along all year.

Furthermore, this study presents the first observation of fin spinelets in one female specimen of *A. altiparanae*. Similar data was described by Fujimoto et al. (2010), whose study dealt with the loach, *Misgurnus anguillicaudatus*. In this species, a bony structure in the pectoral fin, referred to as a bony plate, was supposedly an exclusive phenotypical characteristic of sexually mature males. However, such a structure was found in one wild fish showing an atypical ovary-like gonad. Muñoz et al. (2006) also found something similar in relation to the secondary sexual characteristics of the peacock bass, *Cichla monoculus*. In this study, a single female presented a post-occipital hump, which was thought to be an energy storage structure exclusively associated with spawning capable males (Kelber 1999).

Given the rarity of these structures in females of the abovementioned species, as well as in *A. altiparanae*, these events might be the result of hormonal imbalances. However, this find must be emphasized for *A. altiparanae*, since fin spinelets were previously considered exclusive to males, and had been employed in methods to separate sexes (Garutti and Britiski, 2000; Chehade et al. 2015; Bem et al. 2002; Machado-Evangelista et al. 2019). Thus, the use of this method alone to select for sex can skew the results of experiments and reduce fish farm productivity, since the formed couples might be non-reproductive. The use of other characteristics for said selection is recommended. The animal’s size, for example, can be utilized since almost all the large-sized individuals were female (~94%), with most males being classified as small (~86%). Such a result was already mentioned by Porto-Foresti et al. (2001) for the same species and showed by Sato et al. (2006) for *A. bimaculatus*.

Finally, the reduction in fin spinelet length observed in this study might be related to an initial reduction in estradiol (E2) synthesis caused by gonadectomy. This hormone is produced by Leydig cells in the interstitial compartment of teleosts. Estradiol hormone is thought to positively influence fish secondary sexual characteristics, as demonstrated in guppies (Toft and Baatrup, 2003). In this study, animals incubated in E2 increased their gonopodium in relation to the control group. However, sterility did not influence the presence of fin spinelets in *A. altiparanae*. This finding is similar to the results of Fujimoto et al. (2010) who studied the loach species. The use of external morphological characteristics to certify sterility in fish is desirable, mainly in large-scale sterilization programs, in which 100% of fish sterilization is difficult to achieve (Nozu and Nakamura, 2020). Such a method could speed up selection between sterile and non-sterile animals, avoiding their euthanasia to access the gonad and ensuring the correct animal use in experiments.

In conclusion, this study demonstrated that the presence of fin spinelets in yellowtail tetra (*A. altiparanae*) is a phenotypical secondary sex characteristic influenced by sex, size, and season of the year. These factors appear to have a larger influence than gonadal development. Furthermore, fin spinelets keep growing even within gonadectomized fish, suggesting that these structures are not indicative of fertility in this species.

Acknowledgments The authors are grateful to the Sao Paulo Research Foundation (FAPESP) for the financial support of this research (2010/17429-1 and 2011/11664-1). We also acknowledge CEPTA/ICMBio for generously providing facilities and experimental fish.

Compliance with ethical standards

Conflict of interest The authors declare that they have no conflict of interest.

References

Azevedo MA, Malabarba LR, Fialho CB (2000) Reproductive biology of the inseminating glandulocaudine Diapoma speculiferum Cope (Teleostei: Characidae). Copeia 2000: 983–989

Baldisserotto B (2013) Fisiologia de peixes aplicada à piscicultura: UFSM Santa Maria

Bem JCD, Fontanetti CS, Senhorini JA, Parise-Maltempi PP (2012) Effectiveness of estradiol valerate on sex reversion in Astyanax altiparanae (Characiformes, Characidae). Braz Arch Biol Technol 55(2):283–290
