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Heat-induced triploids in *Brycon amazonicus*: a strategic fish species for aquaculture and conservation

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Summary

Triploidization plays an important role in aquaculture and surrogate technologies. In this study, we induced triploidy in the matrinxã fish (*Brycon amazonicus*) using a heat-shock technique. Embryos at 2 min post fertilization (mpf) were heat shocked at 38°C, 40°C, or 42°C for 2 min. Untreated, intact embryos were used as a control. Survival rates during early development were monitored and ploidy status was confirmed using flow cytometry and nuclear diameter analysis of erythrocytes. The hatching rate reduced with heat-shock treatment, and heat-shock treatments at 42°C resulted in no hatching events. Optimal results were obtained at 40°C with 95% of larvae exhibiting triploidy. Therefore, we report that heat-shock treatments of embryos (2 mpf) at 40°C for 2 min is an effective way to induce triploid individuals in *B. amazonicus*.

Research Article

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Introduction

Fish are the most diverse group of vertebrates with over 34,700 known species (Fricke *et al.*, 2018). More than 6000 of these species inhabit Neotropical freshwater environments. These Neotropical species are highly abundant, making up 20–25% of the global fish population (Reis *et al.*, 2016). However, several factors including pollution, the introduction of invasive species, and riparian forest degradation (Vitule *et al.*, 2009; Barletta *et al.*, 2010; Agostinho *et al.*, 2016; Vitule *et al.*, 2017) are negatively affecting Neotropical ichthyofauna.

These deleterious phenomena specifically affect the genus *Brycon*, with several member species being listed as endangered and under threat of extinction (e.g. *B. orbignyanus*, *B. insignis*, *B. vermelha*, *B. opalinus* and *B. nattereri*) (ICMBio, 2016). Given these circumstances, biotechnical tools, such as germ cell transplantation and fish sterilization, could be important to guarantee genetic conservation (de Siqueira-Silva *et al.*, 2018). Germ cell transplantation (or surrogate technology) involves the generation of a germline chimera through the transference of germ cells from a target species to an appropriate host with the goal of enabling the production of viable gametes from the donor fish (Saito *et al.*, 2008; Lacerda *et al.*, 2010; Nóbrega *et al.*, 2010; Yasui *et al.*, 2010). This technique requires a sterile host so that the transplanted germ cells can develop without endogenous competition (Golpour *et al.*, 2016). Triploidization is one of the main techniques currently utilized to produce sterile hosts for germ cell transplantation (de Siqueira-Silva *et al.*, 2018). The presence of three sets of chromosomes disrupts meiosis, generally making triploid fish sterile (Cuñado *et al.*, 2002). This makes triploid fish suitable hosts for the application of surrogate technology (Okutsu *et al.*, 2007) focused on the preservation of endangered species (Golpour *et al.*, 2016; Goto and Saito, 2019).

Furthermore, the use of triploids also reduces environmental concerns over exotic, transgenic, or hybrid species (Benfey, 2016; do Nascimento, 2017a; Piferrer *et al.*, 2009). For aquaculture purposes, triploid fish can improve growth rates, carcass yields, and meat quality (do Nascimento *et al.*, 2017a, 2017b). Triploidy is commonly induced using chemical, pressure, or temperature shocks (Arai, 2001), which seek to inhibit the process of second polar body extrusion (Dunham, 2004). Another strategy is to use diploid gametes from tetraploid individuals (do Nascimento *et al.*, 2020). However, fertile tetraploids are scarce, difficult to work with (Yoshikawa *et al.*, 2008), and are limited to a few species (Piferrer *et al.*, 2009).

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In Neotropical fish, the induction of triploidy can be applied to several species including *Brycon amazonicus*, also known as matrinxã. *B. amazonicus* is a tropical fish with excellent flesh quality, a rapid growth rate, and high market value. As a result, it is of great aquacultural importance (Gomes, 2010). This species demonstrates migratory behaviour, annual maturation, and total spawning (Zaniboni-Filho *et al.*, 2006). Additionally, *B. amazonicus* is omnivorous and can be easily adapted to captivity (Honczaryk and Inoue, 2009). Therefore, triploid matrinxã may be used to improve aquacultural yields, or to serve as hosts for germ cells from endangered species of the genus *Brycon*. Therefore, the aim of this study was to induce triploidy in *B. amazonicus* using temperature shocks.

Materials and methods

Broodstock induction

Specimens of *B. amazonicus* were maintained in earthen ponds (1000 m^2) at the Centro Nacional de Pesquisa e Conservação da Biodiversidade Aquática Continental/Instituto Chico Mendes de Conservação da Biodiversidade (CEPTA/ ICMBio), Pirassununga City, São Paulo State, Brazil (21°55′58′′S, 47°22′31′′W). The fish were fed twice a day using a commercial diet (4200 kcal kg^{-1} and 45% crude protein).

The experiment was performed during the species' natural spawning season from November to December. Specimens for breeding were captured and selected based on external characteristics. The presence of a swollen abdomen and reddish urogenital papilla was used as a selection criterion for females, while the release of sperm upon the application of gentle abdominal pressure was used as a selection criterion for males. The fish were then carefully transported to spawning tanks with constant water flow. Females ($n = 6$) and males ($n = 6$) were induced to spawn by injection of carp pituitary gland extract (CPE) at 5.5 mg kg^{-1} body weight. The hormone was administered in two different applications for females. The first injection was comprised of 10% of the overall CPE dose. The second injection was conducted 10 h after the first injection and was comprised of the other 90% of the CPE dose. Males received a single dose of CPE (3.0 mg kg^{-1}) at the same time as the second dose for females. When the females began to spawn, the gametes (eggs and sperm) were hand stripped using abdominal massage in separated containers. Fertilization was achieve by immediately mixing the gametes with water from the incubators.

Triploidization

The fertilized eggs were divided into four plastic containers whose bottoms contained a 100-µm nylon mesh. The fertilized eggs were maintained in water at 26°C. At 2 min post fertilization (mpf), three groups of eggs were subjected to heat-shock treatments lasting 2 min at either 38°C, 40°C or 42°C. The fertilized eggs of the fourth container were not subjected to heat-shock treatment and were used as a control group. Immediately following heat shock, all containers were moved to incubators with constant water flow and aeration. These procedures were performed separately for each couple, resulting in six replicates.

Developmental analysis

To conduct an analysis of fertilized eggs during early development, a sample from each treatment group (*c.* 100 eggs) was placed into plastic containers (bottom with 100 µm nylon mesh) at the top of each incubator. Survival rates were then noted at each of the following stages: cleavage, blastula, gastrula, somite, and hatching. Rates of normal and abnormal larvae were also determined (see Figure 1). These procedures were performed with a stereomicroscope (Nikon SMZ 1500,Tokyo, Japan) and accompanying software Nis-Ar Elements (Nikon, Tokyo, Japan). After hatching, samples of 100 larvae from each treatment group were selected for ploidy analysis using flow cytometry.

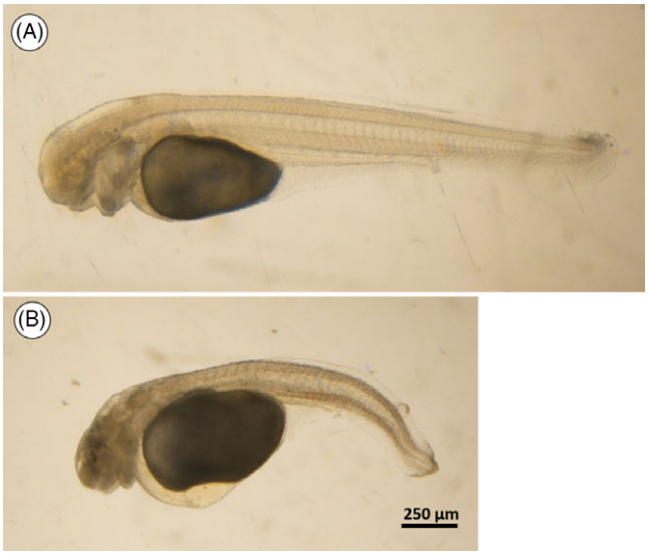

Figure 1. Morphology of normal (*A*) and abnormal (*B*) larvae of *Brycon amazonicus*.

Fish rearing

Larvae from the treatment determined to be optimal were reared until approximately 2 years old. For the first 5 months, the larvae were reared in 5000 litre concrete tanks with constant water flow. During this period, the larvae received commercial powdered food (4200 kcal kg^{-1} and 45% crude protein) five times a day. Afterwards, the fish were transferred to 1000 m^2 earthen ponds, and were fed with commercial pellets (3 mm, 91.62% dry matter, 45% crude protein, 8% crude fat, 2.8% crude fibre, and 12.10% mineral matter) twice a day until apparent satiation.

Flow cytometry

Triploid rates (%) during the larval stage and in adults were detected using flow cytometry according to the protocol developed by Xavier *et al.* (2017). The samples were first lysed in a detergent solution (9.53 mM $MgSO_4.7H_2O$, 47.67 mM KCl, 15 mM Tris, 74 mM sucrose, and 0.8% of Triton X-100). Afterwards, nuclei were stained with 4′,6-diamidino-2-phenylindole dihydrochloride (DAPI). Samples were then analyzed using a flow cytometry CyFlow Ploidy Analyzer (Partec, GMBh, Germany). By comparing histogram peaks, triploid percentages could be determined for each treatment.

Blood smear

Fish (five diploids and five triploid specimens, each *c.* 2 years old) were collected from the maintenance tank and analyzed using flow cytometry as described earlier (see section previously). The animals were anaesthetised in a eugenol solution (1 g l^{-1}) and blood was collected from a caudal puncture using a syringe containing one drop of EDTA (5%). One drop of the collected blood was smeared

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Table 1. Survival (%) of *Brycon amazonicus* in percentage (± SE) after heat-shock treatment for induction of triploid fish

Treatments	Cleavage (%)	Blastula (%)	Gastrula (%)	Somite (%)	Hatching (%)	Normal (%)	Ploidy			
							2n	3n	Mosaic*	3n (%)
Control	86.1 ± 7.5	82.0 ± 12.7	79.4 ± 13.0^{a}	70.9 ± 12.2^{a}	52.8 ± 10.6^{a}	39.0 ± 16.5	100	0	0	0
38	84.4 ± 5.3	83.6 ± 8.1	67.8 ± 10.3^{a}	50.2 ± 8.2^{a}	27.2 ± 7.1^{a,b}	22.2 ± 12.9	95	4	5	0.04
40	78.1 ± 9.9	76.0 ± 16.5	57.2 ± 19.7^{a,b}	36.9 ± 16.4^{a,b}	26.2 ± 14.6^{a,b}	10.3 ± 6.9	5	95	0	95.00
42	72.9 ± 5.5	68.3 ± 7.9	10.1 ± 8.5^{b}	1.3 ± 1.3^{b}	0.0 ± 0.0^{b}	0.0 ± 0.0	–	–	–	–
P-value	0.9656	0.5520	0.0065	0.0018	0.0007	0.1059	–			

Heat shock were applied at 38, 40 and 42°C at 2 mpf for 2 min.
^{a,b}Different superscript letters within a column designates statistical differences using the Tukey test (ANOVA; $P < 0.05$).
Mosaic: fish with 2n and 3n cells; SE: standard error.

using a cover slip by dragging across a slide. The slides were then stained with the rapid panoptic kit (Laborclin, Pinhais, Brazil) and observed with an optical microscope (Nikon NI, Tokyo, Japan) and accompanying software Nis-Ar Elements (Nikon, Tokyo, Japan). The captured images were analyzed using ImageJ software (National Institutes of Health, USA, http://rsb.info.nih.gov/ij/) for measurement ($n = 100$) of area (μm^2), perimeter (μm), major axis (μm), and minor axis (μm).

Statistics

Results are shown as the mean ± standard error. The data were checked for normality using a Lilliefors test. Afterwards, one-way analysis of variance (ANOVA) and a Tukey post hoc test were performed on early developmental data. A *t*-test was applied for erythrocyte measurements. The software STATISTICA v.10.0 (Statsoft, Tulsa, USA) was used with a significance level of $\alpha = 0.05$.

Results

Early development

Data on the development of heat-shocked embryos are shown in Table 1. No difference among treatments were observed for cleavage ($P = 0.9656$), blastula ($P = 0.5520$), or normal stages ($P = 0.1059$). During the gastrula stage ($P = 0.0065$) and somite stage ($P = 0.0018$), the lowest survival rates were observed for fertilized eggs heat shocked at 42°C. Fertilized eggs from this group did not result in any hatching events. Morphology of normal and abnormal larvae are described in Figure 1.

Flow cytometry

Flow cytometry data demonstrated that the control group and fertilized eggs heat shocked at 38°C resulted in mainly diploid individuals (Table 1). However, the group heat shocked at 40°C, resulted in high percentages of triploid individuals (95.00%). A few mosaics were also observed for 38°C (Table 1).

Blood smear

Table 2 summarizes the results obtained from erythrocyte nuclei measurements. Triploid fish showed significantly increased dimensions for area ($P = 0.0001$), perimeter ($P = 0.0001$), major axis ($P = 0.0001$), and minor axis ($P = 0.0001$). Representative examples of blood smears from diploid and triploid individuals are shown in Figure 2.

Table 2. Nuclear measurements of erythrocytes (± SE) from diploid and triploid of *Brycon amazonicus*

Parameter	2n	3n	*P*-value
Area (μm^2)	14.14 ± 0.39^{a}	19.86 ± 0.58^{b}	0.0001
Perimeter (μm)	14.54 ± 0.24^{a}	17.89 ± 0.08^{a}	0.0001
Major axis (μm)	4.99 ± 0.08^{a}	6.18 ± 0.10^{a}	0.0001
Minor axis (μm)	3.61 ± 0.06^{a}	4.06 ± 0.01^{b}	0.0001

SE: standard error.
^{a,b}Distinct letters indicate significant difference (*t*-test; $P < 0.05$).

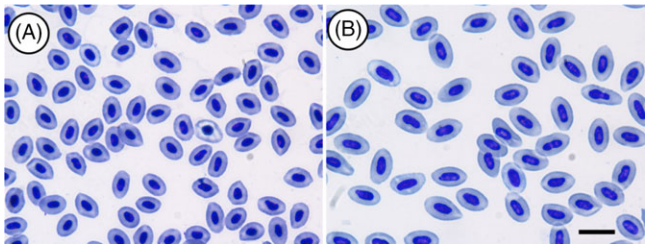

Figure 2. Blood smears of diploid (left) and triploid (right) individuals of *Brycon amazonicus*. Erythrocytes nuclei from diploids (*A*) presented small size in comparison with triploids (*B*). Scale: 10 μm.

Discussion

In this study, high percentages of triploids were obtained for *Brycon amazonicus* using heat-shocked fertilized eggs at 40°C for 2 min. This protocol prevents the extrusion of the second polar body, as observed in previous studies (Adamov *et al.*, 2017; Bertolini *et al.*, 2020; Yasui *et al.*, 2020). In nature, triploid fish can be spontaneously arise, as described in several studies concerning Neotropical fish (Molina *et al.*, 2007; Pansonato-Alves *et al.*, 2011; Utsunomia *et al.*, 2014). However, for aquaculture purposes, triploidy must be induced artificially (Piferrer *et al.*, 2009).

In our study, heat-shock treatments did not guarantee 100% triploidy rate. This is in agreement with previous reports (Adamov *et al.*, 2017; Yasui *et al.*, 2020). This signifies the need to optimize the current protocol or make use of tetraploid individuals (do Nascimento *et al.*, 2020) to enable the mass production (100%) of triploid fish.

Additionally, the larval stage survival rate obtained was lower compared with similar studies (Adamov *et al.*, 2017; Bertolini *et al.*, 2020). The induction of triploid fish may lead to mosaics, as observed here and with Pacific salmon (Teplitz *et al.*, 1994),

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therefore affecting survival rates. Additional research into the formation of mosaics using karyotyping could be interesting in this case.

Erythrocyte measurements have proven to be a simple and inexpensive method for the discrimination of diploid and triploid individuals (Flajšhans *et al.*, 2010; Fukushima *et al.*, 2012; Goo *et al.*, 2015). Using flow cytometry as a validation technique, blood smear analysis was capable of identifying adult triploid individuals of *B. amazonicus*. Therefore, the combination of both tools (erythrocyte nuclei analysis and flow cytometry) offers an accurate procedure for the identification of triploids.

As stated previously, triploid fish have promising aquaculture applications, as they may offer increased carcass yields (%) and improved meat quality (do Nascimento *et al.*, 2017b). However, such fish are also suitable for the conservation of endangered species as they reduce the effect of escaped fish (Dunham, 2004). Another interesting application is the use of sterile triploid fish as hosts for germ cell transplantation studies (Okutsu *et al.*, 2007). Triploids of *B. amazonicus*, for example, could be used to host germ cells from other *Brycon* species threatened with extinction, such as *B. orbignyanus*, *B. insignis*, *B. opalinus*, *B. vermelha* and *B. nattereri* (ICMBio, 2016).

In conclusion, triploidy was efficiently induced in *B. amazonicus* using heat-shock treatments (40°C, 2 min) of fertilized eggs 2 min post fertilization; with 95% of resulting larvae exhibiting triploidy.

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Ethical standards. All procedures were approved by the Ethics Committee by the Guide for the Care and Use of Laboratory Animals of the University of São Paulo (CEUA 4299290819).

Conflict of interest. None.

References

Adamov NSdM, Nascimento NFd, Maciel ECS, Pereira-Santos M, Senhorini JA, Calado LL, Evangelista MM, Nakaghi LSO, Guerrero AHM and Fujimoto T (2017). Triploid induction in the yellowtail tetra, *Astyanax altiparanae*, using temperature shock: tools for conservation and aquaculture. *J World Aquacult Soc* **48**, 741–50.

Agostinho AA, Gomes LC, Santos NC, Ortega JC and Pelicice FM (2016). Fish assemblages in Neotropical reservoirs: colonization patterns, impacts and management. *Fish Res* **173**, 26–36.

Arai K (2001). Genetic improvement of aquaculture finfish species by chromosome manipulation techniques in Japan. *Aquaculture* **197**, 205–28.

Barletta M, Jaureguizar A, Baigun C, Fontoura NF, Agostinho AA, Almeida-Val VMFd, Val AL, Torres RA, Jimenes-Segura LF and Giarrizzo T (2010). Fish and aquatic habitat conservation in South America: a continental overview with emphasis on neotropical systems. *J Fish Biol* **76**, 2118–76.

Benfey TJ (2016). Effectiveness of triploidy as a management tool for reproductive containment of farmed fish: Atlantic salmon (*Salmo salar*) as a case study. *Rev Aquacult* **8**, 264–82.

Bertolini RM, Lopez LS, do Nascimento NF, Arashiro DR, de Siqueira-Silva DH, dos Santos SCA, Senhorini JA and Yasui GS (2020). Strategies for aquaculture and conservation of Neotropical catfishes based on the production of triploid *Pimelodus maculatus*. *Aquacult Int* **28**, 127–37.

Cuñado N, Terrones J, Sánchez L, Martínez P and Santos J (2002). Sex-dependent synaptic behaviour in triploid turbot, *Scophthalmus maximus* (Pisces, Scophthalmidae). *Heredity* **89**, 460–4.

de Siqueira-Silva DH, Saito T, dos Santos-Silva AP, da Silva Costa R, Psenicka M and Yasui GS (2018). Biotechnology applied to fish reproduction: tools for conservation. *Fish Physiol Biochem* **44**, 1469–85.

do Nascimento NF, de Siqueira-Silva DH, Pereira-Santos M, Fujimoto T, Senhorini JA, Nakaghi LSO and Yasui GS (2017a). Stereological analysis of gonads from diploid and triploid fish yellowtail tetra *Astyanax altiparanae* (Garutti & Britski) in laboratory conditions. *Zygote* **25**, 537–44.

do Nascimento NF, Pereira-Santos M, Piva LH, Manzini B, Fujimoto T, Senhorini JA, Yasui GS and Nakaghi LSO (2017b). Growth, fatty acid composition, and reproductive parameters of diploid and triploid yellowtail tetra *Astyanax altiparanae*. *Aquaculture* **471**, 163–71.

do Nascimento NF, Pereira-Santos M, Levy-Pereira N, Monzani PS, Niedzielski D, Fujimoto T, Senhorini JA, Nakaghi LSO and Yasui GS (2020). High percentages of larval tetraploids in the yellowtail tetra *Astyanax altiparanae* induced by heat shock: the first case in Neotropical characins. *Aquaculture* **520**, 734938.

Dunham R (2004). *Aquaculture and Fisheries Biotechnology: Genetic Approaches*. CABI. ISBN 0-85199-596-9.

Flajšhans M, Gela D, Kocour M, Buchtová H, Rodina M, Pšenička M, Kašpar V, Piačková V, Sudová E and Linhart O (2010). A review on the potential of triploid tench for aquaculture. *Rev Fish Biol Fish* **20**, 317–29.

Fricke R, Eschmeyer W and Van der Laan R (2018). Catalog of fishes: genera, species, references. California Academy of Sciences, San Francisco, CA, USA http://researcharchive.calacademy.org/research/ichthyology/catalog/fishcatmain.asp.

Fukushima H, Bailone R, Weiss L, Martins M and Zaniboni-Filho E (2012). Triploidy in the hematology of jundia juveniles (Siluriformes: Heptapteridae). *Brazil J Biol* **72**, 147–51.

Golpour A, Siddique MAM, Siqueira-Silva DH and Pšenička M (2016). Induced sterility in fish and its potential and challenges for aquaculture and germ cell transplantation technology: a review. *Biologia* **71**, 853–64.

Gomes LC (2010). Matrinxã (*Brycon amazonicus*). In (ed. LCGB Baldisserotto) *Espécies Nativas para Piscicultura no Brasil*, pp. 608–91.

Goo IB, Im JH, Gil HW, Lim SG and Park I-S (2015). Comparison of cell and nuclear size difference between diploid and induced triploid in marine medaka, *Oryzias dancena*. *Dev Reprod* **19**, 127.

Goto R and Saito T (2019). A state-of-the-art review of surrogate propagation in fish. *Theriogenology* **133**, 216–27.

Honczaryk A and Inoue LAKA (2009). Produção comercial de alevinos de matrinxã na Amazônia Ocidental. Embrapa Amazônia Ocidental-Circular Técnica (INFOTECA-E).

ICMBio (2016). *Sumário Executivo do Livro Vermelho da Fauna Brasileira Ameaçada de Extinção*. ICMBio Brasilia.

Lacerda SM, Batlouni SR, Costa GM, Segatelli TM, Quirino BR, Queiroz BM, Kalapothakis E and França LR (2010). A new and fast technique to generate offspring after germ cells transplantation in adult fish: the Nile tilapia (*Oreochromis niloticus*) model. *PLoS One* **5**, e10740.

Molina WF, Margarido VP and Galetti Jr PM (2007). Natural triploidy in *Leporinus* cf. *elongatus* bearing sex chromosomes. *Genet Mol Biol* **30**, 567–9.

Nóbrega RH, Greebe CD, Van De Kant H, Bogerd J, de França LR and Schulz RW (2010). Spermatogonial stem cell niche and spermatogonial stem cell transplantation in zebrafish. *PLoS One* **5**, e12808.

Okutsu T, Shikina S, Kanno M, Takeuchi Y and Yoshizaki G (2007). Production of trout offspring from triploid salmon parents. *Science* **317**, 1517.

Pansonato-Alves JC, Oliveira C and Foresti F (2011). Karyotypic conservatism in samples of *Characidium* cf. *zebra* (Teleostei, Characiformes, Crenuchidae): physical mapping of ribosomal genes and natural triploidy. *Genet Mol Biol* **34**, 208–13.

Piferrer F, Beaumont A, Falguiere J-C, Flajšhans M, Haffray P and Colombo L (2009). Polyploid fish and shellfish: production, biology and applications to aquaculture for performance improvement and genetic containment. *Aquaculture* **293**, 125–56.

https://doi.org/10.1017/S0967199421000125 Published online by Cambridge University Press

Reis R, Albert J, Di Dario F, Mincarone M, Petry P and Rocha L (2016). Fish biodiversity and conservation in South America. *J Fish Biol* **89**, 12–47.

Saito T, Goto-Kazeto R, Arai K and Yamaha E (2008). Xenogenesis in teleost fish through generation of germ-line chimeras by single primordial germ cell transplantation. *Biol Reprod* **78**, 159–66.

Teplitz R, Joyce J, Doroshov S and Min B (1994). A preliminary ploidy analysis of diploid and triploid salmonids. *Can J Fish Aqua Sci* **51**, 38–41.

Utsunomia R, Pansonato-Alves JC, Paiva LRS, Costa Silva GJ, Oliveira C, Bertollo LAC and Foresti F (2014). Genetic differentiation among distinct karyomorphs of the wolf fish *Hoplias malabaricus* species complex (Characiformes, Erythrinidae) and report of unusual hybridization with natural triploidy. *J Fish Biol* **85**, 1682–92.

Vitule JRS, Freire CA and Simberloff D (2009). Introduction of non-native freshwater fish can certainly be bad. *Fish Fish* **10**, 98–108.

Vitule J, Da Costa A, Frehse F, Bezerra L, Occhi T, Daga V and Padial A (2017). Comment on 'Fish biodiversity and conservation in South America by Reis et al. (2016)'. *J Fish Biol* **90**, 1182–90.

Xavier PL, Senhorini JA, Pereira-Santos M, Fujimoto T, Shimoda E, Silva LA, dos Santos SA and Yasui GS (2017). A flow cytometry protocol to estimate DNA content in the yellowtail tetra *Astyanax altiparanae*. *Front Genet* **8**, 131.

Yasui GS, Fujimoto T and Arai K (2010). Restoration of the loach, *Misgurnus anguillicaudatus*, from cryopreserved diploid sperm and induced androgenesis. *Aquaculture* **308**(Supp 1), S140–4.

Yasui GS, Nakaghi LSO, Monzani PS, Nascimento NFd, Pereira dos Santos M, Goes CAG, Porto-Foresti F and Senhorini JA (2020). Triploidization in the streaked prochilod *Prochilodus lineatus* inferred by flow cytometry, blood smears and karyological approaches. *J Appl Ichthyol* **36**, 336–41.

Yoshikawa H, Morishima K, Fujimoto T, Arias-Rodriguez L, Yamaha E and Arai K (2008). Ploidy manipulation using diploid sperm in the loach, *Misgurnus anguillicaudatus*: a review. *J Appl Ichthyol* **24**, 410–4.

Zaniboni-Filho E, Reynalte-Tataje D and Weingartner M (2006). Potencialidad del género *Brycon* en la piscicultura brasileña. *Rev. Colomb de Cienc Pecu* **19**, 233–40.

https://doi.org/10.1017/S0967199421000125 Published online by Cambridge University Press